Using OCT image to distinguish human acupoint from non-acupoint tissues after irradiation with laser *in vivo*: a pilot study

Huiqing Zhong (钟会清)[1], Zude Zhang (张祖德)[1], Zhouyi Guo (郭周义)[1], Huajiang Wei (魏华江)[1*], Hongqin Yang (杨洪钦)[2], Yonghong He (何永红)[3], Shusen Xie (谢树森)[2], and Songhao Liu (刘颂豪)[1]

1 Key Laboratory of Laser Life Science of Ministry of Education of China, South China Normal University, Guangzhou 510631, China

2 Key Laboratory of Optoelectronic Science and Technology for Medicine of Ministry of Education of China, Fujian Normal University, Fuzhou 350007, China

3 Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China

[*]E-mail: weihj@scnu.edu.cn

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Using the optical coherence tomography (OCT) images, the optical attenuation coefficients (μ_t) of human *Laogong* acupoint and non-acupoint tissues are measured after empty irradiation and 808-nm 100-mW irradiation for 10 min *in vivo* non-invasively. The results show that there is no significant difference of μ_t of *Laogong* acupoint and non-acupoint tissues after empty irradiation. However, there are significant differences of μ_t between *Laogong* acupoint and non-acupoint tissues after laser irradiation at the power of 100 mW (statistical definition of probability $p < 0.01$). The results of the pilot study indicate that the OCT could distinguish the acupoint from the surrounding tissues after irradiation with laser *in vivo* non-invasively.

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Optical coherence tomography (OCT) is a relatively new non-invasive optical diagnostic technique that provides depth-resolved images of tissues with resolution up to a few micrometers and depths up to several millimeters (depending on tissue type). This technique has been extensively applied in many field of biomedicine[1−4] since its introduction in 1991[5].

Meridian theory is an important part of traditional Chinese medicine for thousands of years. Acupuncture is based on the principle of establishing equilibrium by either upward or downward adjustment of the functional and energy states of the human body. Therefore, acupuncture is a convenient and effective therapy for some diseases[6−8]. Despite acupuncture's long history and tradition, it is difficult for biomedical scientists to search for the specific constituents of human acupuncture and meridian because of the complexity of the human body structure. In order to reveal the secret of acupuncture and meridian system, some hypotheses on neurology, anatomy, and physiology were proposed during the past years. However, the exact acupoint and the differences between acupoint and non-acupoint were still uncertain. Therefore, many scientists began to study the physical properties of human acupuncture meridians *in vivo* in the viewpoint of their physiological function. There are some technologies used to stimulate acupuncture points in all ages, such as stone, metal, and electro-acupuncture (i.e., the application of small electric currents through the inserted needles), and recent developments of laser acupuncture technology have already been introduced[9]. The laser acupuncture was defined as the stimulation of traditional acupuncture points with low-intensity and non-thermal laser irradiation[10]. Although laser acupuncture has been accepted widely, the nature of the meridian system and the principles of laser acupuncture have not been fully explained by modern science.

Several studies have reported about investigating human acupuncture and meridian system by using modern scientific methods, such as electrical impedance measurement[11−14], infrared (IR) thermal imaging[15,16], IR spectrum analysis[17,18], and functional magnetic resonance imaging[19]. However, these methods only non-invasively revealed the existence of human meridian and its properties to some extent, which limited the accuracy on distinguishing acupuncture points from their surrounding tissues. Recently, it was reported that using a system could obtain accurate control over the measurement position and fine tuning statistical analysis of the results[20], yet with a poor resolution of 1 mm. Spectrometric study was also performed at the acupoint tissue after laser irradiation[21].

Based on these studies, we propose a non-invasive OCT system to observe the optical difference of human acupoint and non-acupoint tissues *in vivo* under near-infrared (NIR) laser irradiation. This method is simple with high resolution and can be performed on other acupoints with good reproducibility.

Eleven healthy volunteers (six males and five females) were recruited from different departments of South China Normal University (mean ± standard deviation (SD) age is 23.3±3.0 years). The subjects had no history of chronic diseases and were healthy at the time of enrolment. All subjects agreed to participate in the study and offered the written informed consent. The experimental protocol was approved by the Lab of Photonic Chinese Medicine, Key Laboratory of Laser Life Science of Ministry of Education and Institute of Laser Life Science, South China

Normal University, and South China Normal Hospital.

The OCT system was an extension of Michelson interferometer implemented by a low-coherence light source[1,22]. The broadband light source of the OCT system was a super-luminescent diode with the central wavelength at 1310 nm and a bandwidth of 50 nm. This OCT system provided an axial resolution of 10 – 15 μm. The transverse resolution of the system was about 25 μm, which was determined by the focal spot size produced by the probe beam. It was a non-invasive imaging system (with power of 0.1 – 1 mW). The signal-to-noise ratio (SNR) of the system was measured to be 100 dB. A visible light source (λ = 645 nm) was used to guide the probe beam. The OCT system operation was controlled automatically by a computer. Figure 1 shows the schematic diagram of the OCT system. Each in-depth scanning (A-scan) consisted of 10000 data points. Lateral scanning (B-scan) image was obtained by moving the mirror relative to the tissue sample, which took about 1.0 s. Two-dimensional (2D) OCT images were obtained in each experiment and stored in computer for further processing[23]. The 2D OCT images were averaged in the lateral direction (x-axis) (which was sufficient for speckle-noise suppression) into a single curve to obtain a one-dimensional (1D) OCT signal that represented 1D distribution of light in-depth scale.

The relative humidity of the room was about 55% ± 10%. All subjects were performed in a dark room, and the room temperature was kept at 25 ± 2 °C during OCT imaging measurements. The subjects were allowed to stabilize for at least 30 min before commencing recording in the room. The location of the *Laogong* acupoint (PC-8) and the non-acupoint were labeled before the experiment with the help of an acupuncturist. Non-acupoint was about 2 cm away from PC-8. The power of the NIR laser used for irradiation was 100 mW. The power of the near-infrared laser used for irradiation was 100 mW, which is the power of the laser resource, and there is a 2-mm pinbole diameter between fiber and measured point. The power density of laser in the measured point is 200 mW/cm^2. The irradiation time was 10 min for PC-8 or non-acupoint. In order to reduce the measurement error, the interval between PC-8 and non-acupoint measurements was six days (PC-8 was measured by OCT system after empty irradiation (0 mW) on the first day, and was measured immediately after irradiated at the power of 100 mW on the second day. The procedures for non-acupoint were performed after six days). All experiments were performed at the same time every day.

OCT imaging is based on the difference of backscatter light. In this model, OCT is assumed to detect light

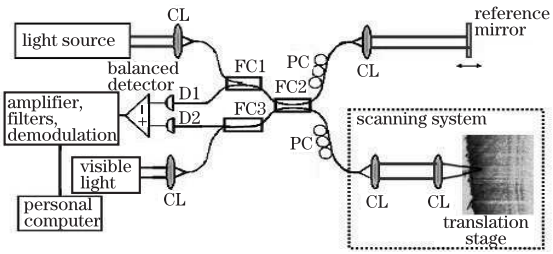

Fig. 1. Schematic of the OCT system. FC: fiber coupler; PC: polarization controller; OL: objective lens; D: detector; CL: collimating lens.

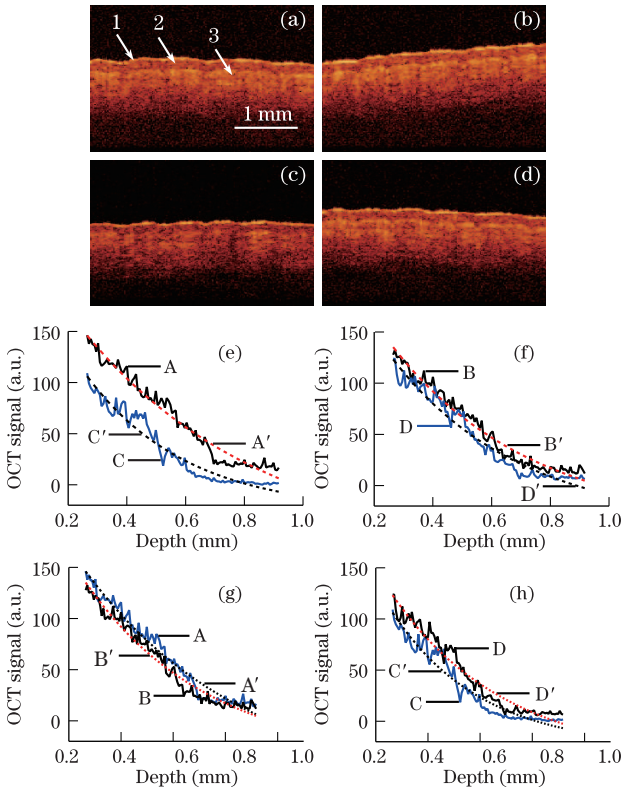

Fig. 2. (a) and (b) are OCT images of PC-8 and non-acupoint after empty irradiation. (c) and (d) are OCT images of PC-8 and non-acupoint after irradiated at the power of 100 mW. (e) Corresponding OCT signals (A, C) and corresponding fit curves (A′, C′) of tissue in the dermis area from (a) and (c). (f) Corresponding OCT signals (B, D) and corresponding fit curves (B′, D′) of tissue in the dermis area from (b) and (d). (g) is the OCT signal and corresponding fit curves of PC-8 (A, A′) and non-acupoint (B, B′) after empty irradiation. (h) is the OCT signals and corresponding fit curves of PC-8 (C, C′) and non-acupoint (D, D′) after irradiated at the power of 100 mW. The horizontal and vertical axes (depth) of (a), (b), (c), and (d) present the imaging lateral length (in millimeters) and the imaging depth (in millimeters), respectively. 1, stratum corneum; 2, epidermis; 3, dermal layer and area under dermis.

that has only scattered once, and thus the decay of the OCT signal with depth function follows the Beer-Lambert law. According to the Beer-Lambert law, light attenuation inside tissues is exponential. The total attenuation coefficient is μ_t ($\mu_t = \mu_s + \mu_a$, where μ_s is the scattering coefficient and μ_a is the absorption coefficient). Because $\mu_a \ll \mu_s$ for tissues in the NIR spectral range, only the backscattered components from the tissue contribute to the OCT image[24,25]. By analyzing the exponential profile of light attenuation (1D OCT), we can obtain information on tissue scattering properties. If the scattering coefficient changes, as a result, the 1D OCT signal will change. Therefore, the differences of PC-8 and non-acupoint after irradiated by laser at the same power can potentially be detected with the OCT system from μ_t. With a Levenberg-Marquardt curve fitting algorithm[23], we could get

$$i(d) = A\exp(-\mu_t d) + y_0, \quad (1)$$

where i is the signal, d is the penetration depth of the OCT images, A is the scaling factor, and y_0 is the offset.

Table 1. Comparison of the Curve Fit Relative Values ($\bar{x} \pm s$) of OCT Signal Intensity

Groups	Case(n)	R^2	μ_t (mm^{-1})	$\Delta\mu_t$ (mm^{-1})
PC-8 (empty irradiation)	11	0.969	1.615 ± 0.194	
PC-8 (100 mW)	11	0.965	2.918 ± 0.350	1.302*↑
Non-Acupoint (empty irradiation)	11	0.957	2.103 ± 0.252	
Non-Acupoint (100 mW)	11	0.970	2.274 ± 0.273	0.1704

* represents $p < 0.05$, ↑ indicates the up-regulation of μ_t.

Equation (1) was fitted to the OCT signal within and under the dermis area of skin.

The data were represented by ± SD. The results were analyzed using SPSS13.0 software t-tests. The statistical definition of probability $p < 0.05$ stood for significant difference.

Figures 2(a) and (b) show the OCT images of PC-8 and non-acupoint after empty irradiation, and Figs. 2(c) and (d) show the OCT images of PC-8 and non-acupoint after laser irradiation of 808 nm for 10 min at the power of 100 mW. It can be seen that the OCT images of PC-8 and non-acupoint after irradiated at the power of 100 mW become darker than those after empty irradiation, and there is a more obvious change in the OCT images of PC-8 when compared with those of non-acupoint. In order to further analyze the difference between the OCT images of PC-8 and non-acupoint, OCT signals fit exponential curves as functions of depth are quantitatively plotted in Figs. 2(e) and (f) ($p < 0.05$). The results show that there is a significant difference between empty irradiation and 100-mW irradiation in μ_t of PC-8, and the difference between empty irradiation and 100-mW irradiation in μ_t of non-acupoint is not obvious compared with that of PC-8. Figures 2(g) and (h) show the OCT signals and corresponding fit curves of PC-8 and non-acupoint ($p < 0.01$). From Fig. 2(g), it can be clearly seen that there is no significant difference between PC-8 and non-acupoint after empty irradiation in μ_t, but from Fig. 2(h), the difference in μ_t between PC-8 and non-acupoint after 100-mW irradiation is obvious compared with that after empty irradiation.

Using Eq. (1) for fitting statistics ($p < 0.05$), the correlation coefficients R^2 of PC-8 and non-acupoint after irradiated at the power of 100 mW are shown in empty irradiation and Table 1. It means that they fit the original well. The increase of μ_t (1.302) of PC-8 is more than that (0.1704) of non-acupoint (comparing 100-mW with empty irradiation).

In conclusion, we have presented a simple and non-invasive experimental method for studying the differences of μ_t between human PC-8 and non-acupoint tissues. The experiments are non-invasive after empty irradiation and 808-nm 100-mW irradiation for 10 min. Our study suggests that the increase of μ_t of PC-8 is larger for the acupoint than that of non-acupoint after laser irradiation. These results demonstrate the capability of OCT technique to distinguish acupoint from non-acupoint. This method would be very helpful in further research of specificity of acupoint and clinical applications for laser acupuncture.

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