Application to skin physiology using optical coherence tomography

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Background and aims: The sweat glands and peripheral vessels beneath the skin surface act as minute organs governed by the skin sympathetic nerves and have important physiological functions for body temperature control and nutrition support along with maintenance of a peripheral organization. Dynamics of the mental sweating of sweat glands and the peripheral vessels reflect the activity of a sympathetic nerve. The purpose of this paper is to study the dynamic observation and analysis of sweat glands and a peripheral vessels by optical coherence tomography (OCT).

Materials and Methods: In the dynamic analysis of mental sweating of sweat glands, after confirmation of the resting state of the volunteer, mental stress was applied in the form of unpleasant sound for 0.5 sec; piled-up en-face OCT images of sweat glands were then obtained time-sequentially, with the frame-spacing of 3.3 sec. A swept-source (SS) OCT was used for in vivo en-face OCT of a group of sweat glands on the subject's fingertip. Furthermore, we conducted in vivo dynamic analysis in response to external mental stress of a peripheral vessel in the second joint of the subject's third finger using 1.3-µm SS OCT.

Results: We analyzed time variation in the amount of excess sweat produced by a group of sweat glands and found a large difference in the amount of sweat stored by each sweat gland in the spinal lumen. Mental stress was also shown to cause the small artery of the finger to contract, reducing blood flow. In particular, the thickness of the tunica media of the small artery changed abruptly in response to the sound stress, increasing and then decreasing so that the artery contracted and expanded, respectively.

Conclusions: Dynamic analysis of mental sweating in the eccrine sweat glands and changes in peripheral vessels was performed using time-sequential OCT imaging. For mental sweating, time variation in the amount of excess sweat produced could be simultaneously evaluated for a few tens of eccrine sweat glands. Furthermore, we performed the dynamic analysis of a peripheral vessel in a human finger in response to external mental stress and found that the small artery contracted and expanded in response to sound stress while continuing to pulse in synchronization with the heartbeat. These studies have the potential for establishing new knowledge about skin physiology.

Key words: optical coherence tomography (OCT) • skin physiology • mental sweating • sweat gland • small artery • sympathetic nerve

1. Introduction

Optical coherence tomography (OCT) is an epoch-making noninvasive technique for imaging biological tissue, which can provide clear tomographic images with a spatial resolution of 10–20 µm at a depth of a few millimeter beneath the skin surface [1, 2]. OCT is now used for clinical diagnoses of macular degeneration of retina diseases in ophthalmology and arteriosclerosis in circulatory medicine [3, 4]. In addition to the clinical applications, OCT has been used in brain research by Maheswari et al., who attained instantaneous OCT signals of the nerve response to a stimulus in the cerebral cortex of a cat [5].

Recently, we demonstrated the use of dynamic OCT for skin physiology, including investigating the dynamics of sweat glands and peripheral vessels...
2. Materials and methods

2.1 Sweat glands and peripheral vessels underneath the skin controlled by the sympathetic nerve

In human perspiration or sweating, there are two types of sweating, thermal and mental sweating [13]. Mental sweating is stimulated not only by physical stress such as a firm hand grip or catching a ball but also by mental stress such as sound stress or being required to perform mental arithmetic. The nerve center that detects stress is closely connected to the premotor area of the cerebral cortex, the limbic cortex, and the hypothalamus. Excess sweating due to stress is controlled via the acetylcholine receptor by impulses from the nerve center. The amount of excess sweating, therefore, directly reflects activity of the sympathetic nerves.

Conversely, if the subject is astonished by an external stress, he or she may behave as if defending himself or herself from an aggressor. In this physiological response, the blood flow into skeletal muscles increases abruptly to enable the defense, whereas peripheral vessels, such as those of the fingers, contract to reduce blood flow. The nerve center that detects stress is closely connected to the premotor area of the cerebral cortex. Contraction of the vessel wall due to the external stress is controlled via noradrenalin receptors by impulses from the nerve center [14]. In particular, a small artery continuously pulses in synchronization with the heartbeat and expands or contracts in response to external stress. Therefore, the response of the peripheral vessels also reflects the activity of the sympathetic nerve as well as mental sweating.

2.2 Simultaneous observation of mental sweating of a group of sweat glands using the swept-source OCT (SS-OCT)

In the epidermis beneath the skin surface of the human fingertip, eccrine sweat glands coil up to absorb sweat from the surrounding tissues. In OCT images, eccrine sweat glands in the stratum corneum of the epidermis are clearly recognizable as spiral lumens with a high reflection light intensity due to the large difference in refractive index at the wall of the spiral lumen between sweat and the keratinous epidermal tissue. Exact evaluation is possible in three-dimensional (3D) images of the eccrine sweat gland, where the 3D image is constructed by volume rendering of OCT images (Fig. 1). In the experiment, swept-source (SS) OCT (Thorlabs OCM 1300SS) was used for in vivo 3D imaging of eccrine sweat glands, with an image resolution of 15 µm at 25 frames/s with a central wavelength of 1.325 µm. The sweeping wavelength of the light source was 100 nm at a frequency of 16 kHz and with a coherence length of 6.0 mm. The volume rendering was performed using commercially available software (Amira 4.1.0 Resolve RT, Mercury Computer Systems, Inc.). The image size was 0.2 × 1.0 mm², with the pixel number of 32 × 171. The 3D image was constructed by volume rendering of 32 B-mode OCT images with a pixel size of 6.25 µm along the y-direction.

In the dynamic analysis of mental sweating, the piled-up en-face OCT images were obtained time-sequentially, simultaneously tracking as many sweat glands as possible. The image construction method for the en-face OCT of a group of sweat glands is shown.
in Fig. 2. The eccrine sweat glands align along the fingerprint on a human fingertip are shown in Fig. 2a. The B-mode OCT image in the $x$–$z$ plane was obtained where the image size was $2.0 \times 1.5$ mm$^2$ with a pixel size of $12.5 \times 5.9$ µm$^2$. We then eliminated the bright line that indicated the skin surface and the deep layer in the dermis; these cause undesirable background noise in the OCT image. A total of 128 OCT images in the $x$–$z$ plane were obtained with a spacing of 12.5 µm over a distance of 1.6 mm along the $y$-direction (Fig. 2b). The imaging time was 3.3 s, which was the frame spacing of the time-sequential piled-up en-face OCT images. From the 128 B-mode OCT images, the en-face OCT images in the $x$–$y$ plane were extracted with a slice spacing of 5.9 µm (Fig. 2c). All en-face OCT images were piled up to evaluate the amount of excess sweat. In the resulting piled-up en-face OCT image (Fig. 2d), the amount of excess sweat could be evaluated simultaneously for 17 eccrine sweat glands aligned along four lines of the fingerprint on a human fingertip.

Dynamic OCT was performed to investigate the mental sweating of a group of eccrine sweat glands on an area of $2.0 \times 1.6$ mm$^2$ on the middle fingertip of a 23-year-old male volunteer. After confirmation of the resting state of the volunteer, mental stress was applied to the volunteer using headphones in the form of an unpleasant sound of breaking glass for 0.5 s with a sound level of 90 dB at the sound frequency of about 4000 to 5000 Hz. The piled-up en-face OCT images of sweat glands were obtained with a frame spacing of 3.3 s as shown in Fig. 3. In the active sweat glands, marked by a white circle in the figure, the reflection light intensity increased suddenly in response to the sound stress. In our previous study, the amount of sweat stored in the sweat glands could be evaluated quantitatively for a given instant by summation of the reflection light intensity of all pixels included in the

Fig. 2: Construction method of the en-face OCT images of the sweat gland. (a) Fingerprint on a human fingertip; (b) 128 OCT images in the $x$–$z$ plane are obtained with a spacing of 12.5 µm along the $y$ direction; (c) the en-face OCT images in the $x$–$y$ plane are extracted with the slice spacing of 5.9 µm; (d) The piled-up en-face OCT image.

Fig. 3: Time-sequential piled-up en-face OCT images for dynamic analysis of several sweat glands.
spiral lumen. Figure 4 shows the time variation of the reflection light intensity for the sweat gland, corresponding to the amount of sweat produced in response to the sound stress. Figure 4a shows the piled-up en-face OCT image after 62.7 s, and Figure 4b and 4c shows the time variation of the reflection light intensity for the sweat glands from A3 and A4, respectively. These two sweat glands were active, but there was a large difference in the amount of the stored sweat in the spiral lumen. This showed that the response to mental stress was different for each sweat gland even though the sweat glands were aligned with each other.

We also evaluated the time-integrated value for the reflection light intensity over the 200 s after application of the sound stress; this corresponds to the total excess sweat produced. Figure 5 shows an example of such integration for a sweat gland, where the integration was performed over the average value of the signal intensity of the resting state. Another example of time integration of the reflection light intensity is also shown (Fig. 6). From these results, considerable non-uniformity was observed in mental sweating, with the amount of excess sweat produced in response to the sound stress being different for each sweat gland. Such strong non-uniformity with mental sweating should be necessary to adjust the total amount of excess sweat in response to the strength of the stress.

**Fig. 4:** Time variation of the reflection light intensity of the sweat gland, corresponding to the amount of sweat in response to the sound stress. (a) The piled-up en-face OCT image after 62.7 s, (b) and (c) are time variation of the reflection light intensity for the sweat glands from A3 and A4.

**Fig. 5:** An example of integration of reflection light intensity of the sweat gland according to the excess sweat.
2.4 Dynamic imaging of the peripheral vessels of a human finger by SS-OCT

Peripheral vessels are distributed in the dermis of human skin. OCT has particular potential for dynamic analysis of the blood microcirculation system, including small arteries and veins, arterioles, venules, capillary vessels, and arteriovenous anastomoses that lie 1-2 mm beneath the skin surface. SS OCT (santec, HSL-2000) was used for *in vivo* imaging of the peripheral vessels of a finger, with an image resolution of 12 m at 25 frames/s and a central wavelength of 1.324 m. The sweeping wavelength of the light source was 110 nm at a frequency of 20 kHz with a coherence length of 9.2 mm. Such a long coherence length is desirable for imaging deep tissue. In the experiment, clear OCT imaging was performed successfully near the proximal interphalangeal joint of a volunteer’s third finger (a 23-year-old male). The OCT image of the small artery of the third finger is shown (Fig. 7), where the image size was $2 \times 2 \text{ mm}^2$, equivalent to $512 \times 512$ pixels. There are three layers around a small artery: the tunica intima, tunica media, and tunica adventitia. The tunica media, in particular, can be recognized as a black band in the OCT image; it is composed of five to six muscle layers. During actual imaging, insertion of a 2-mm-thick glass plate behind an object brings a shift of nearly 1 mm in the focal plane. The finger was pressed against the glass plate with a pressure of 50 mmHg; under this condition, the small artery to be imaged was

![Fig. 7](image)

*Fig. 7:* *In vivo* OCT image of a small artery underneath the skin surface of a human finger.
elliptic in shape.

In the next experiment, sound stress was applied to the subject after confirmation of his resting state. Again, the stress was an unpleasant noise (the sound of breaking glass) played for 0.5 s at a sound level of 90 dB. The response of a small artery in the finger to sound stress was tracked by time-sequential OCT images at 25 frames/s, which allowed dynamic observation of the artery. Figure 8 shows time-sequential OCT images of the small artery every 5 s; the sound stress was applied at $t = 30$ s. Immediately after the sound stimulus, the lumen of the small artery contracted gradually, and the cross section of the lumen of the small artery (lumen is depicted in Fig. 7) reached its minimum around $t = 40$ s. It then expanded gradually to recover its resting state. We were able to measure the cross-sectional area of the small artery as well as the thickness of the tunica media. Figure 9 shows measurement of the thickness of the small artery from the OCT image. The small artery including tunica media and the lumen were framed by the region of interest (ROI) (Fig. 9a), and the raster signals were averaged as shown (Fig. 9b), with a standard deviation of 0.74 µm. Figure 10 shows the time variation of the thickness of the tunica media (red line) and the area of the small artery (blue line) in response to the sound stimulus. In particular, we can find that the pulse waves of the small artery with heartbeat. Three seconds after the stimulus, the tunica media started becoming thicker, reaching its maximum around 12 s and then contracting gradually during 30–40 s. Increases and decreases of the tunica media contract or expand the small artery, respectively. The tunica media thickness increased about 60 µm. During this response

Fig. 8: Experimental protocol and time-sequential OCT images of the small artery with the dynamic motion in response to sound stimulus, where the blue area shows the period of construction of the small artery.
of the small artery, it continued to pulse in synchronization with the heartbeat.

4. Discussion And Conclusions

We performed dynamic analysis of mental sweating of eccrine sweat glands and peripheral vessels by time-sequential OCT imaging. In the analysis of mental sweating, we were able to evaluate time variation of the amount of excess sweat simultaneously in a few tens of eccrine sweat glands. These imaging techniques can be applied to evaluate the activity of sympathetic nerves and any abnormalities. We also conducted dynamic analysis of a peripheral vessel of a human finger in response to external mental stress and found that the small artery contracted and expanded in response to a sound stress while continuing to pulse in synchronization with the heartbeat. These studies have the potential for establishing new knowledge of skin physiology.

In this study, we performed 1.3-μm swept-source OCT imaging of internal microstructures beneath the skin. For tissue imaging, 1.3-μm light is more useful than 0.8-μm light for deep penetration because of scat-
tering by biological tissue. In a SS-OCT imaging system, the A-mode scanning rate is determined by the repetition rate of the swept light laser, and the imaging depth is determined by the coherence length of the light source. Currently, swept light sources are being developed worldwide; in particular, sources with a 10-mm long coherence length are being developed by the optical MEMS 15).

Our efforts are directed at the improvement of OCT imaging techniques such as high-speed image acquisition and high-resolution OCT imaging, leading to more precise construction of 3D OCT images. A detailed discussion of the dynamic analysis of minute organs will appear elsewhere.

**Ethical concerns**

Before experimental procedure, we obtained written informed consents from all volunteers. This study was conducted under approval from the ethics review board of Osaka University Graduate School of Medicine (No. 96-1).

**Disclosure of possible conflict of interests**

No conflict of interest exists.

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**References**


