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2018

Yu, X., Tang, H., Hu, C., Ding, Q., Wang, L., Wang, X., . . . Liu, L. (2018). Multiscale skin imaging in vivo using optical coherence tomography. *Laser Physics Letters*, 15(7), 075601-.
doi:10.1088/1612-202X/aabb2f

<https://hdl.handle.net/10356/137387>

<https://doi.org/10.1088/1612-202X/aabb2f>

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Multiscale skin imaging *in vivo* using optical coherence tomography

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1 April 2018

Abstract. Optical coherence tomography (OCT) is a noninvasive high-resolution diagnostic imaging modality that plays an increasingly important role in dermatology. Diagnosis of skin diseases using OCT requires both cellular-level high resolution and large area skin coverage. In practice, however, there exists a trade-off between the achievable spatial resolutions and the transverse scanning range. In this study, we report a Micro-OCT (μ OCT) system that is capable of providing three-dimensional (3D) images of the skin at multiple spatial scales with both cellular-level resolution ($1 \sim 2 \mu m$) mode and large area ($\sim 20 \times 20 mm^2$) scanning mode. Specifically, in the cellular-level scanning mode, we achieve a transverse resolution of $\sim 1.5 \mu m$ and an axial resolution of $1.7 \mu m$ ($n = 1.38$) which enables the visualization of cellular-level skin microstructures. While in the large-area scanning mode, the system is capable of covering an *en face* imaging area reaching up to $20mm \times 20 mm$ with a lateral resolution of $\sim 5.5 \mu m$ at a scanning speed of $60K$ A-lines/s. We experimentally verify the imaging capabilities of such a multiscale μ OCT system including *in vivo* visualization of epidermal cells in the cellular-level scanning mode as well as the internal fingerprints and sweat gland ducts in the large area scanning mode. Micro-anatomical imaging at multiple spatial scales could provide comprehensive information of the skin that is valuable to disease diagnosis.

PACS numbers: 42.15.Eq, 42.62.Be, 87.85.Ox, 87.85.Pq

Keywords: Optical Coherence Tomography, Imaging systems, Medical and biological imaging, Medical optics instrumentation. Submitted to: *Laser Phys. Lett.*

1. Introduction

Optical coherence tomography (OCT) is a powerful noninvasive imaging modality based on low-coherence interferometry and Confocal reflectance microscopy [1]. By measuring the magnitude and echo time delay of the backscattered signals from the sample and the reference arm optics, OCT is able to provide cross-sectional images of the microstructures of biological tissues with typical axial resolutions of $1 \sim 15 \mu\text{m}$ [2]. Due to its noninvasive and high-resolution properties, OCT has not only been established as a routine clinical tool for ophthalmic imaging, but also been utilized in some other areas, e.g., skin imaging [3], airway function evaluation [4], cardiology [5], and gastroenterology [6, 7], etc.

Among those application areas, skin imaging is one of the major fields that have attracted extensive growing research interests in recent years, and the OCT systems, especially high-resolution OCT that is capable of providing more detailed information of the tissue micro-structures at or even below the cellular-level, have been widely applied in dermatology [3]. One of the most important applications of OCT is for skin morphology imaging, e.g., the tumor identifications and skincare products effect evaluation, and numerous studies have been reported in literature. Through utilizing the commercial high-definition OCT (HD-OCT) (Skintell®; Agfa, Belgium), Boone *et al.* and Maier *et al.* evaluated the feasibility of OCT for both basal cell carcinoma detection [8–10] and melanocytic lesions identification first [11–13], and then adopted the HD-OCT for actinic keratosis visualizations with different scanning models [14, 15] as well as actinic keratosis discriminations from normal skin and squamous cell carcinoma using an identification method [16]. The HD-OCT systems have also been applied for both intrinsic skin ageing assessments in women and reaction visualization to permanent make-up [16, 17].

Skin functional imaging, including the inflammatory condition monitoring and cutaneous glucocorticosteroid atrophy effect evaluation, etc., is another one of the most important areas for OCT applications in dermatology. Specifically, Boone *et al.* assessed the possibility of differentiating the allergic and irritant contact dermatitis with HD-OCT using a proposed pattern recognition algorithm [18], and also evaluated the applicability of OCT for real-time 3D assessment of epidermal splitting and decellularization [19], while H.L. Tey *et al.* studied the applicability of HD-OCT for the diagnoses of both morphea [20] and miliaria profunda in clinical practice [21]. In addition, OCT systems have also been utilized in some other areas, such as wound healing monitoring [22], facial region vascular malformations [23] and extracellular matrix and skin fibrosis distribution [24], etc., in dermatology.

However, it is worth mentioning that all those studies rely mainly on the commercial HD-OCT systems with resolutions of $\sim 3 - 15 \mu\text{m}$ and $\sim 4.5 - 24 \mu\text{m}$ in axial and lateral directions, respectively. Although such high-resolutions help visualize more detailed microstructures of the skin tissue, the relatively small scanning area of such system hinders their applications in clinical practice for skin imaging. Such is because skin is

the largest organ of the human body and the disease lesions may spread over a large (square centimeters) area, and therefore, it is usually troublesome and time-consuming to locate the diseased areas accurately, which finally may lead to missed diagnoses or misdiagnoses. To address such issues with OCT skin imaging and facilitate its applications in clinical practice, it is imperative to develop OCT systems with both cellular-level resolution and large scanning range.

In this study, we demonstrate a compact, multiscale Micro-OCT (μ OCT) system that provides a viable solution to the above-mentioned problem by integrating a large area scanning mode and a cellular-level scanning mode into a single system, and such a μ OCT achieves both higher resolutions and larger *en face* scanning area than the commercial HD-OCT systems for skin structure imaging *in vivo*. With the broadband light source employed, an axial resolution of $\Delta z = 1.7 \mu m$ ($n = 1.38$) is achieved in its two scanning modes. While by exchanging the magnification power of the objective lenses in different operation modes, a multiscale scanning function is realized. Specifically, with the cellular-level scanning mode, a high lateral resolution of $\Delta \delta = \sim 1.5 \mu m$ is achieved, while with the large area scanning mode, an *en face* imaging area reaching up to $20 mm \times 20 mm$ is achieved with the lateral resolution slightly decreased to $\delta = \sim 5.5 \mu m$ in air. Both the system performances and its ability for *in vivo* skin imaging are characterized experimentally.

2. Methods

2.1. Imaging system construction

The construction of the μ OCT imaging system is depicted in Fig. 1(a). A superluminescent diode (SLD) array (Superlum Broadlighters T-850-HP) with a center wavelength of $\lambda_c = 850 nm$ and a full width at half maximum (FWHM) bandwidth of $\Delta \lambda = 165 nm$ was employed as the light source. Its output power was measured to be $20.3 mW$. The source output was split into two paths by a broadband 50:50 fiber coupler (TW850R5A2, Thorlabs Inc.). The light directed to the reference arm was first collimated by lens L1 (AC080-020-B-ML, Thorlabs Inc.) and then passed through a UV fused silica window (#49-643, Edmund Optics Inc.), which was utilized to compensate the dispersion introduced by the path length difference between the reference and sample arm optics, before being focused by lens L2 (identical to objective lens L4) to reference mirror M1. The sample arm optics followed the exact path length as that of the reference arm with a galvo scanner (GVSM002/M, Thorlabs Inc.) added in between the collimation lens L3 and the objective lens L4 for sample scanning. The optical power illuminating on the sample was measured to be $7.38 mW$ in this study. The collimation lens L3 together with the galvo scanner and the objective L4 constituted a handheld probe as depicted in Fig. 1(b). Specifically, on such a handheld probe, a silica glass window that is identical to the one in the reference arm was placed after L4 with its back surface fixed around $50 \mu m$ above the focal plane. With such a configuration, the

sample light was approximately focused at the epidermal layer when the silica window is in direct contact with the skin. By interchanging the objective lenses with different magnification powers, the system could switch between cellular-level scanning mode and large-area scanning mode freely. In this study, both L2 and L4 were equipped $f = 10 \text{ mm}$ lenses (M Plan Apo NIR 20X, Mitutoyo Inc.) in cellular-level scanning mode, and were replaced by $f = 50 \text{ mm}$ lenses (AC254-050-B-ML, Thorlabs Inc.) in large-area scanning mode.

The light signals backreflected from the reference arm and those backscattered from the sample arm were recombined by the fiber coupler to generate interferometric signals and finally were guided to a customized spectrometer. Such a spectrometer was comprised of a collimation lens L5 (AC127-030-B-ML, Thorlabs Inc.), a diffraction grating (1200 l/mm @ 830nm, Wasatch Photonics Inc.), a camera lens (Nikon AF Nikkor 85mm f/1.8D), and a line scan CCD camera (E2V, AViiVA EM4). The spectrometer efficiency, including the grating diffraction efficiency, sensor quantum efficiency, and camera lens efficiency, was measured to be 0.416 in experiments. The detected signal was transferred to a computer through camera link cables and an image acquisition card (KBN-PCE-CL4-F, Bitflow Inc.) at 12-bit digital resolution. In the experiments, both scanning camera and galvo scanners were synchronized by a triggering signal generated by the computer. The effective camera pixel number covered by the laser spectrum was measured to be 1322 at FWHM.

Figure 1. Experimental setup of the demonstrated μ OCT system. (a) The detailed μ OCT imaging system configuration. (b) The portable handheld imaging probe. PC: polarization controller; SMF: single-mode-fiber; L1-5: achromatic lens; L6: camera lens; W: UV fused silica window; M1: reference mirror, IMAQ: image acquisition system.

2.2. Multiscale μ OCT system scanning function

To switch between the cellular-level scanning mode and the large-area scanning mode, it is necessary to exchange the pair of objective lenses, i.e., L2 and L4 in reference arm and sample arm optics, with different focal lengths. In the traditional OCT systems, however, it is troublesome to rebuild the whole sample and reference arm optics for mode switching, since it may introduce dispersion effects due to the path length mismatching.

In this study, we designed a compact handheld probe as shown in Fig. 2 to facilitate mode switching. As shown in Figs. 2(a) and 2(b), such a probe consists of two main parts, i.e., an objective lens holder in Fig. 2(a) and a galvo scanner box in Fig. 2(b), and each part has 8 magnets such that the objective lens holder and the galvo scanner box could be connected together. Once there is a need to exchange μ OCT scanning mode, such magnetic pairs allows the two probe parts to be disconnected for objective lens exchange, which thus helps save the operation time in clinical practice.

Figure 2. A compact handheld probe design for convenient μ OCT scanning mode switching. (a) The objective lens holder. (b) The customized galvo scanner box to hold galvo scanners. Red arrows: magnets equipped for μ OCT scanning mode switching.

2.3. System performance characterization

The magnification powers of the sample arm optics were (2X) and (1/2.5X) with the $f = 10 \text{ mm}$ and $f = 50 \text{ mm}$ objective lenses, respectively, and therefore, the corresponding system lateral resolutions were predicted to be $\sim 1.5 \mu\text{m}$ and $\sim 7.36 \mu\text{m}$ in cellular-level scanning mode and large-area scanning mode. While the theoretical axial resolution was calculated to be $\Delta_z = 1.93 \mu\text{m}$ in air for the SLD light source with a center wavelength of $\lambda_c = 850 \text{ nm}$ and a FWHM bandwidth of $\Delta\lambda = 165 \text{ nm}$.

We verified the system transverse resolving power by imaging the 1951 USAF resolution chart. Fig. 3(a) depicts an *en face* image of the resolution chart acquired in cellular-level scanning mode, which consists of $512 \text{ pixels} \times 512 \text{ pixels}$, covering an area of $0.26 \text{ mm} \times 0.26 \text{ mm}$. As can be seen, the line pattern of group 7 element 6 with a line spacing of $2.19 \mu\text{m}$ were resolved clearly. Such imaging result demonstrated that the lateral resolution of the constructed μ OCT system is much finer than $2.19 \mu\text{m}$. In this study, we estimated the system lateral resolution to be $\sim 1.5 \mu\text{m}$ in cellular-level scanning mode. While to characterize the system axial resolution, we placed an actuated iris diaphragm (SM05D5, Thorlabs Inc.) with an attenuation of 20.4 dB into the sample arm optics, and measured the A-line profiles by placing a BK7 prism (with an attenuation of 14 dB) at the focal plane of the sample arm optics. Fig. 3(b) depicts

the measured axial point spread functions (PSFs) at different path length differences for a single A-line, and results demonstrate that the system axial resolution is measured to be $\sim 2.3 \mu\text{m}$ in air.

Figure 3. Spatial resolution characterization of the constructed μOCT in cellular-level scanning mode. (a) An *en face* image ($512 \text{ pixels} \times 512 \text{ pixels}$, $0.26 \text{ mm} \times 0.26 \text{ mm}$) of the 1951 USAF resolution chart. (b) The measured axial PSFs of a single A-line at different path length differences.

We adopted the same procedure to characterize the resolving capabilities of the system in large-area scanning mode. Fig. 4(a) depicts an *en face* image of the resolution chart covering an area of $13.1 \text{ mm} \times 13.1 \text{ mm}$. As can be seen, the line pattern of group 5 element 6 with a line spacing of $8.77 \mu\text{m}$ were clearly resolved. Specifically, when reducing the scanning area to $1.3 \text{ mm} \times 1.3 \text{ mm}$, the finer line patterns as shown in Fig. 4(b) were observed, wherein the finer line pattern of group 6, element 4 with a line spacing of $5.52 \mu\text{m}$ can be well resolved. The profiles for both vertical and horizontal bars of such a line pattern are depicted in Fig. 4(c). Such results demonstrate that the system lateral resolving power reaches up to $\sim 5.5 \mu\text{m}$ in the large-area scanning mode. Furthermore, with a BK7 prism (with an attenuation of 14 dB) placed on the focal plane and an actuated iris diaphragm (with an attenuation of 20.4 dB) inserted into the sample arm optics, the signal intensities of a single A-line at different path-length differences between the sample and the reference arm optics were measured. When normalized with respect to that at a path length difference of $\sim 20 \mu\text{m}$, the obtained normalized signal intensities in decibel (dB) with increasing path-length differences are shown in Fig. 4(d). Results in Fig. 4(d) indicated that the system 6 dB sensitivity rolling-off happens at a path length difference of $\sim 0.48 \text{ mm}$. In addition, since the signal-to-noise ratio (SNR) was measured to be 54.6 dB at the path length difference of $\sim 100 \mu\text{m}$, we estimated the system sensitivity to be $\sim 105.9 \text{ dB}$, which was slightly lower than the theoretical value of 107.5 dB.

Figure 4. Spatial resolution characterization for constructed μ OCT system in large-area scanning mode. (a) *En face* image of the 1951 USAF resolution chart with a scanning area of $13.1 \text{ mm} \times 13.1 \text{ mm}$. (b) A “zoom-in” *en face* image of the 1951 USAF resolution chart with a scanning area of $1.3 \text{ mm} \times 1.3 \text{ mm}$. (c) The averaged cross-sectional profile image for both horizontal and vertical bars of group 6, element 4. (d) The measured axial PSFs of an A-line signal at different path-length differences between the sample and the reference arm optics, showing the sensitivity rolling-off of the μ OCT system.

3. Results

3.1. Cellular-level scanning mode with 10 mm objective lens

To further verify the system imaging capability, human skin tissue at the forearm were imaged *in vivo* in the cellular-level scanning mode. The imaging protocol was reviewed and approved by the institutional review boards at Nanyang Technological University (NTU). Specifically, in the imaging process, a skin gel (Aquasonic 100, Parker Laboratories Inc.) was applied in between the skin and the glass window at the handheld scanning probe to solve the refractive index mismatching problem before imaging. While in the imaging process, the probe was placed in contact with the imaging area without any motion until the data set was collected.

Figure 5 (a) presents a typical cross-sectional image of human skin tissue at forearm

in vivo. Such an image consists of $501 \text{ pixels} \times 488 \text{ pixels}$, covering an area of $0.85 \text{ mm (W)} \times 0.26 \text{ mm (D)}$. As can be seen, the detailed skin micro-structures, such as the stratum corneum, epidermis layer, papillary dermis as well as the dermis layer can be resolved due to the system high spatial resolution in both axial and lateral directions. Specifically, as shown in Fig. 5(a), the sub-cellular level epidermal cells, which appear to be black dots due to the non-reflective nuclei, were also observed as marked by the yellow arrows. With all those acquired cross-sectional images, *en face* images of the skin tissue could be extracted from the reconstructed volumetric data. Fig. 5(b) presents an *en face* image of the acquired skin tissue at an imaging depth of $35 \mu\text{m}$ (from the SC surface). The image consists of $501 \text{ pixels} \times 400 \text{ pixels}$ covering an area of $0.85 \text{ mm} \times 0.68 \text{ mm}$. Again, it can be observed that both stratum corneum and the sub-cellular level epidermal cells could be resolved clearly. Such imaging results convincingly demonstrate that the constructed μOCT system is capable of providing both cellular- and sub-cellular level tissue microstructure images with its cellular-level scanning mode.

Figure 5. Skin tissue at the forearm acquired *in vivo* with the cellular-level scanning mode. (a) A typical cross-sectional image showing the microstructures of the tissue. (b) The *en face* image of the acquired skin tissue at an imaging depth of $35 \mu\text{m}$ (from the SC surface). Both cellular-level layered structures, e.g., stratum corneum, epidermis, papillary dermis (PD) and dermis, as well as the sub-cellular structure, e.g., epidermal cell nucleus, could be resolved. Yellow arrows: epidermal cell nucleus; SC: stratum corneum, PD: papillary dermis.

3.2. Large-area scanning mode with 50 mm objective lens

We tested the system imaging performances in large-area scanning mode by imaging human thumb at the fingertips *in vivo* using $f = 50 \text{ mm}$ objective lens. Fig. 6 presents a typical cross-sectional image of the fingertip at a volunteer thumb *in vivo*. Such an image consists of $3,072 \text{ pixels} \times 1,310 \text{ pixels}$, covering an imaging area of $13.1 \text{ mm (W)} \times 1.1 \text{ mm (D)}$. Imaging results showed that the layered tissue structures, e.g., the epidermis and dermis layers, were clearly resolved; meanwhile, the detailed skin

morphology features, such as the stratum corneum, the spiral sweat ducts as well as the dermal papillae, etc., could also be clearly visualized as marked in Fig. 6.

Figure 6. A typical cross-sectional image of the human thumb at the fingertip acquired *in vivo* in large-area scanning mode. Both the epidermis and dermis layers could be clearly resolved; the detailed morphology features, e.g., stratum corneum (SC), spiral sweat ducts (SD) as well as dermal papillae (DP), were also visualized. .

With a number of consecutive cross-sectional 2D images acquired, the volumetric 3D image of the fingertip tissue could be obtained, and meanwhile, the *en face* images of the fingertip could also be extracted. Fig. 7(a) presents a representative reconstructed 3D volumetric image of the fingertip, which consists of $3072 \times 3072 \times 1380$ pixels covering a volume of 20 mm (L) $\times$ 20 mm (W) $\times$ 1.2 mm (D) in x, y, z direction, respectively. As can be seen, the fingerprint furrow pattern together with the sweat ducts (marked with yellow arrows) distributed along the furrows could be visualized clearly. To provide a clearer vision of those spiral sweat ducts, Fig. 7(b) depicts a small portion of the volumetric 3D image with a volume of 13.1 mm (L) $\times$ 13.1 mm (W) $\times$ 1.2 mm (D). The imaging results in Fig. 7(b) showed that fingerprint furrow pattern together with the individual spiral sweat glands along such furrow pattern could be clearly resolved. When further extract the *en face* image from the volumetric image shown in Fig. 7(b) at an imaging depth of 50 μm (from the stratum corneum), the image as shown in Fig. 7(c) could be obtained. Such an *en face* image shows that the subsurface fingerprints could be obtained with both furrow pattern and the sweat ducts clearly resolved, which again indicates that a μOCT system could be utilized for reliable fingerprint detections [25]. These imaging results obtained with the large-area scanning mode also indicates that the μOCT system could help locate the desired imaging area quickly for diagnosis in clinical practice.

4. Discussions and conclusion

We report a multiscale μOCT system that integrates both cellular-level scanning mode and large-area scanning mode together for skin imaging *in vivo* in this study.

Figure 7. Fingertip tissue imaging with large-area scanning mode. (a) 3D volumetric image of the fingertip tissue showing the imaging capability of the μOCT system. (b) Enlarged 3D image of the selected area showing the individual spiral sweat ducts. (c) An *en face* image (at a depth of $50\text{ }\mu\text{m}$) of the fingertip tissue. FFP: fingertip furrow pattern; SD: sweat ducts. Yellow arrows: individual sweat duct resolved; red arrow: showing selected area.

Experimental results demonstrate that such a $\sim\text{OCT}$ system can not only be used to scan large tissue areas with standard resolution, but also could “zoom in” at smaller areas of interest (AOI) to visualize the cellular-level structural details. Specifically, with the cellular-level scanning mode, the system is capable of providing images of the detailed cellular-level layered structures, e.g., stratum corneum, epidermis, papillary dermis (PD) and dermis, and the sub-cellular level microstructures, e.g., epidermal cell nucleus. While with the large-area scanning mode, the μOCT system could visualize a large volumetric tissue up to 20 mm (L) $\times$ 20 mm (W) $\times$ 1.2 mm (D). Therefore, it is expected that the μOCT system could be utilized to screen through the whole targeted skin area for lesions quickly with large-area scanning mode, while to confirm the findings at much finer granularity with the cellular-level scanning mode. Such a convenient multiscale mode switching function could help largely improve the diagnostic accuracy with reduced workload and operation time. Furthermore, as such a μOCT is able to obtain the human fingertip microstructure details, e.g., fingerprint, individual sweat duct, etc., at and beneath skin, it is also of potential significance to fingerprint-based security applications and forensic science [25, 26].

Some limitations, however, were still observed with the demonstrated μOCT system. One of the major issues was the limited scanning speed. In our experiments, the scanning speed was set to be 60K A-lines/s , which was not high enough for practical applications, especially for *in vivo* and large area scanning applications, since it would make the imaging process time-consuming and prone to motion artifacts. To address such a limited speed issue, the high-speed swept-source OCT (SS-OCT) could be a

potential candidate. Another issue was the limited software processing speed to obtain the 3D volumetric tissue images, especially when the scanning area is large. Future development of the multiscale μ OCT system will be improving the imaging acquisition and processing speed to reduce the motion artifacts, while incorporating a fast lens slider to reduce the system down time during mode switch. In addition, the development of appropriate image processing and evaluation mechanism is also of critical importance to make the imaging results convincing to clinicians.

In conclusion, we have designed and validated a multiscaling imaging solution to an unmet clinical problem. Our proposed multiscale μ OCT imaging system was able to visualize the epidermal nuclei with the cellular-level scanning mode and acquire volumetric skin images over a large en face area of 20 mm $\times$ 20 mm in large-area scanning mode. One can switch between two modes quickly by exchanging the objective lens on a compact handheld probe. The proposed imaging method is simple and can be easily implemented in clinical suites.

Acknowledgments

This research is supported in part by the National Natural Science Foundation of China (Grant No. 61705184), National Research Foundation Singapore (NRF-CRP13-2014-05), Ministry of Education Singapore (MOE2013-T2-2-107), National Medical Research Council Singapore (NMRC/CBRG/0036/2013), and NTU-AIT-MUV program in advanced biomedical imaging (NAM/15005).

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