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# Structural characterization on *in vitro* porcine skin treated by ablative fractional laser using Optical Coherence Tomography

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## ABSTRACT

Ablative fractional skin laser is widely applied for various skin conditions, especially for cosmetic repairing and promoting the located drug delivery. Although the influence of laser treatment over the skin has been explored before in means of excision and biopsy with microscopy, these approaches are invasive, only morphological and capable of distorting the skin. In this paper the authors use fresh porcine skin samples irradiated by the lasers, followed by detected by using Optical Coherence Tomography (OCT). This advanced optical technique has the ability to present the high resolution structure image of treated sample. The results shows that laser beams can produce holes left on the surface after the irradiation. The depth of holes can be affected by changes of laser energy while the diameter of holes have no corresponding relation. Plus, OCT, as a valuable imaging technology, is capable of monitoring the clinical therapy procedure and assisting the calibration.

**Keywords:** Optical Coherence Tomography (OCT), Ablative fractional skin laser treatment

## 1. INTRODUCTION

Ablative fractional laser (AFL) is an invasive skin treatment modality. A main laser beam is divided into several micro-beam zones. Each of the micro-beam has a diameter of a few hundred-micron meter and targets a tiny fractional range of skin at a time. The theoretical principle for this new therapeutic treatment is that laser beam induces micron-level vertical holes, surrounded by a slim layer of coagulated tissue, which composes the microscopic treatment zones (MTZ). AFL damages the target region for specific therapy while adjacently non-irradiated skin has the ability to remodel the skin without scarring [1][2]. Primary applications are broadly involved in cosmetic field, especially for dermal remodeling in the therapy of photo-damaged facial skin, burning scar recovery and etc. Besides, AFL could theoretically facilitate the uptake of topically applied drugs, for instance, promoting the incubation time of the photosensitizer and enhance penetration depth of drugs as ablative laser holes could reach dermis layer, thereby acting as channel for drug uptake [3][4]. The volume of the holes induced by laser have significant influence over the efficacy of the therapeutic process. In clinical, shallow holes are generated for the treatment of superficial skin conditions while the deep holes are specific to scar remodeling, and the depth is also magnificently dependent on the location for the drug delivery treatment [5]. Even higher energy might harm the skin, like burning or scarring. Up to date, there is a few literatures studying the size of holes generated by AFL *in vivo*, but most of the papers are concentrating on the research where tissues are excised and the outcomes are presented from the view of microscope [6][7]. This post-treatment with most possibility produces the tissues distorted and therefore contributes no precise measurement.

Non-invasive imaging techniques like magnetic resonance imaging (MRI) and ultrasound (US) have similar ability to provide tissue structure reconstruction. However, both of these two imaging methods are just capable of performing in tens to hundreds of microns scale, which is failed to give high resolution and limit their effectiveness using in detecting the condition of the superficial dermis layer. Optical Coherence Tomography (OCT) [8] is an advanced optical imaging technique that has the potential to produce high-resolution, cross-sectional image of tissue structure in micron level. It is also a non-invasive, *in vivo* and enables to generate images in real time. The basic principle of OCT system is similar to the Michelson interferometer, based on the interference between the sample and reference light beam [9]. This novel imaging technology has a wide range, such as medical diagnosis and surgical guidance in clinical application and characterization of objects with multi-layers. Furthermore, OCT system is considered as a reliable measurement tool to

supply quantitative imaging detection, which has a magnificently impact on the fields like tissue engineering and polymer composite manufacture.

The study in this paper illustrates an application of the OCT system to detect how AFL treatment affects the geometry of porcine skin sample. To explore the difference induced by AFL, the porcine skin samples were radiated by laser with different energy. After that, treated samples were detected by FD-OCT to observe the structure.

## 2. MATERIAL AND METHOD

### 2.1 Sample preparation

The porcine skin samples were whittled from fresh pork belly slices bought at local slaughter store. All samples were stored in PBS and the experiment was performed within 8 hours. The scalpel was used for trimming and each sample is approximately 20 mm in length, 20 mm in width and 5mm in depth. The samples contain the epidermis, dermis and fat layer and were thick enough for the laser treatment. Once all the samples were placed into petri dish fulfilled with PBS, followed by storing in foam box filled with ice to maintain the fresh. Samples were brought to Photobiology Unit (NHS Tayside, Ninewells Hospital) for AFL irradiation then conducted OCT scanning in OCT lab in Ninewells Hospital.

### 2.2 System configuration

In this experiment, a carbon dioxide laser machine and a smoke evacuator were involved in the process of laser irradiation and OCT system was used for structure reconstruction. The laser beam (**Alma Pixel CO<sub>2</sub>**) is divided into 9×9 array shown in Figure 1(a) and the energy of each pixel was control from the terminal. A smoke evacuator (**ACU-EVAC II Surgical Smoke Evacuator**) was applied to extract the smoke produced during the irradiation. Figure 1(b) displayed the setup and the operation of laser irradiation, which was completed by the staff charging of laser treatment in Photobiology department. The energy used in this study ranged from 100 mJ/pixel to 350 mJ/pixel with 50 mJ/pixel interval. The laser energy is the product of power and radiated time and power that is fixed for each laser model, meaning that the energy of each laser beam is only the function of irradiated time.

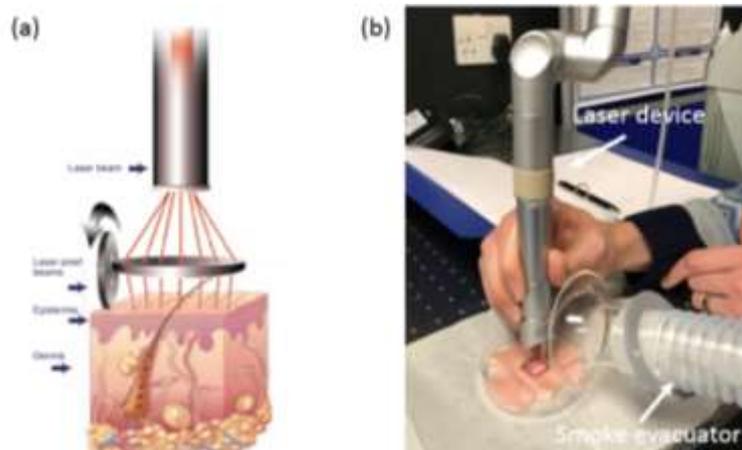


Figure 1. (a) The schematic diagram of laser beam array [10]. (b) The experiment setup of laser irradiation treatment

In this study, a phase sensitive optical coherence tomography (PhS-OCT) was applied to achieve the structure observation [11][12]. This optical imaging system (Figure 2) was implemented by a spectral domain configuration. The light source is supplied by a superluminescent diode with a central wavelength of 1310 nm and a bandwidth of 85 nm. The detection light on the sample was delivered by an objective lens of 30 mm focal length along the detector arm. The acquisition rate was mainly determined by the spectrometer camera which was set on the frequency of 46 KHz. The axial and lateral resolution was 8.9  $\mu\text{m}$  and 15  $\mu\text{m}$  respectively.

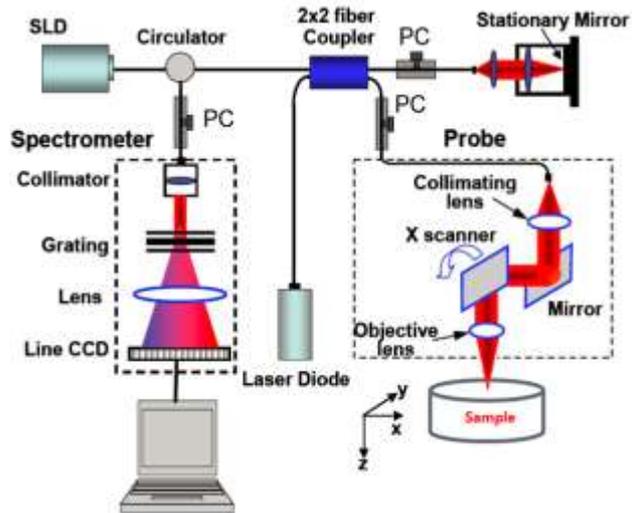


Figure 2. The schematic diagram of phase sensitive optical coherence tomography (PhS-OCT)

### 2.3 Imaging protocol

In OCT scanning procedure, three-dimensional structural and two-dimensional structure images were acquired. For a cross-sectional 2D structure image, the OCT beam accomplishes 512 A-line scans repeatedly at each spatial location sequentially completing a whole B-scan (total 512 locations). Thus, a B-scan contains 512×512 A-scans. For the 3D structural image, a B-scan only with one A-scan at one location was achieved at one cross sectional position and then 512 B-scans were completed from one side to the other side. Therefore, one 3D structural image contains 512 B-scans and each B-scan has 512 A-scans.

## 3. RESULTS

As OCT system is capable of producing high resolution two-dimensional structural images, we explored how the shape of the holes have been affected with the increase of laser energy. Figure 3(a) shown the post-treatment condition that 9×9 holes distributed over the surface after the laser irradiation. In Figure 3(b), the cross-sectional image clearly presented the geometry of the holes (red circle) and a three-dimensional structure image was reconstructed in Figure 3(c). To characterize the influences over porcine skin under varied laser energy, two parameters: depth and width were induced to qualitatively describe the variations. From Figure 4(a) to Figure 4(f), samples treated by laser beam with energy from 100 mJ to 350 mJ were demonstrated with 50 mJ interval. It could be seen that the holes were becoming deeper with the increase of laser energy. Another phenomenon that could be observed is that the treated areas have higher scattering intensity, causing most of light reflected from the surface and forming the shadow region beneath the holes.

Figure 5(a) displayed hole depth after the treatment with different laser energy. Under the minimum laser energy (100 mJ) irradiation, the depth of holes in were less than 0.05 mm. With the raise of treatment energy, the depths were approximately 0.09 mm, 0.115mm and 0.13 mm under 150 mJ, 200 mJ and 250 mJ treatment respectively. When the laser energy increased to 300 mJ, holes depth reached the maximum, greater than 0.16 mm. However, the depth of hole shrank with the maximum energy treatment in this study.

The width (diameter) of hole was also treated as another key parameter to depict the geometry change. Figure 5(b) illustrated the changes of the hole width under the laser treatment with the energy from 100 mJ to 350 mJ. From the chart, it was found that the diameter of the holes on the surface were independent on the laser treatment energy. There was no rising or falling trend happened on hole width change with the raise of laser energy, and hole width values were fluctuating between 0.1 mm and 0.3 mm.

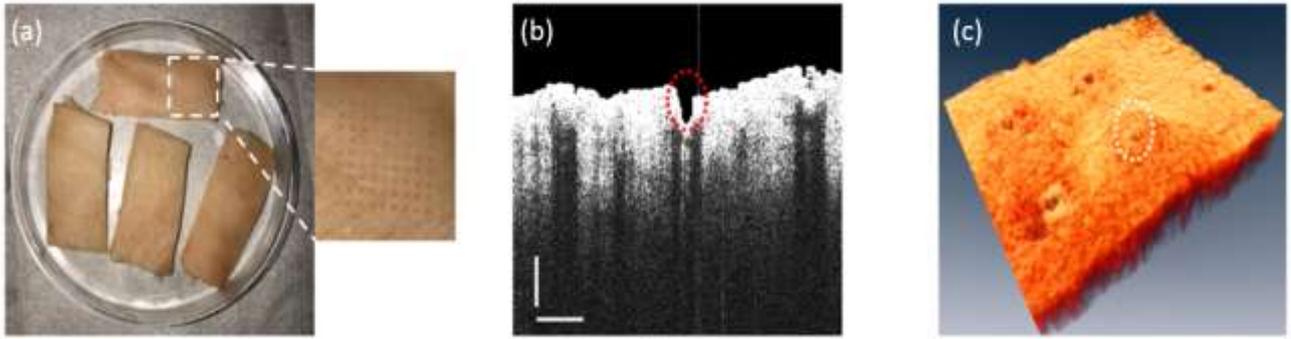


Figure 3. (a) Porcine skin sample after laser treatment. (b) Cross-sectional structure image. Red circle region is the hole left after irradiation. The vertical and horizontal scale bars are  $200\mu\text{m}$  and  $500\mu\text{m}$  respectively. (c) 3-D structure image.

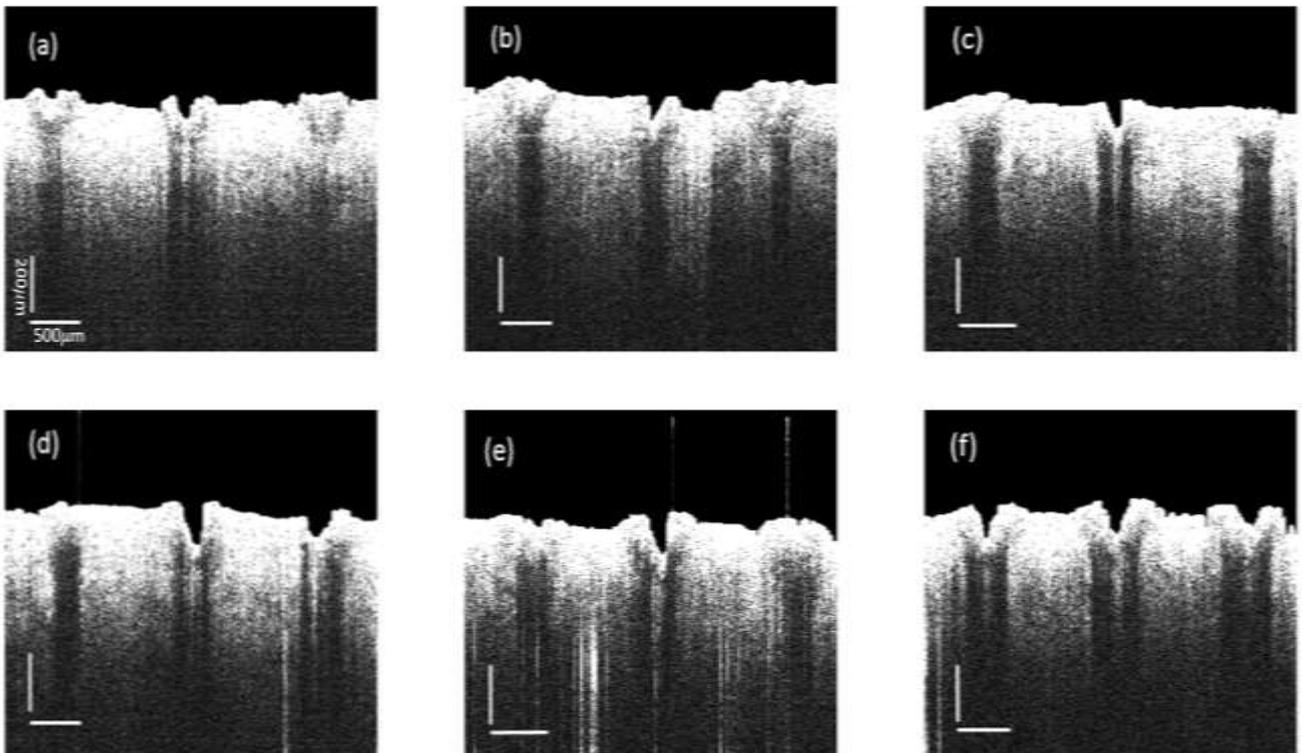


Figure 4. The cross-sectional structure images (a)-(f) of the porcine skin samples treated by laser beam with energy from  $100\text{mJ}$  to  $350\text{mJ}$ .

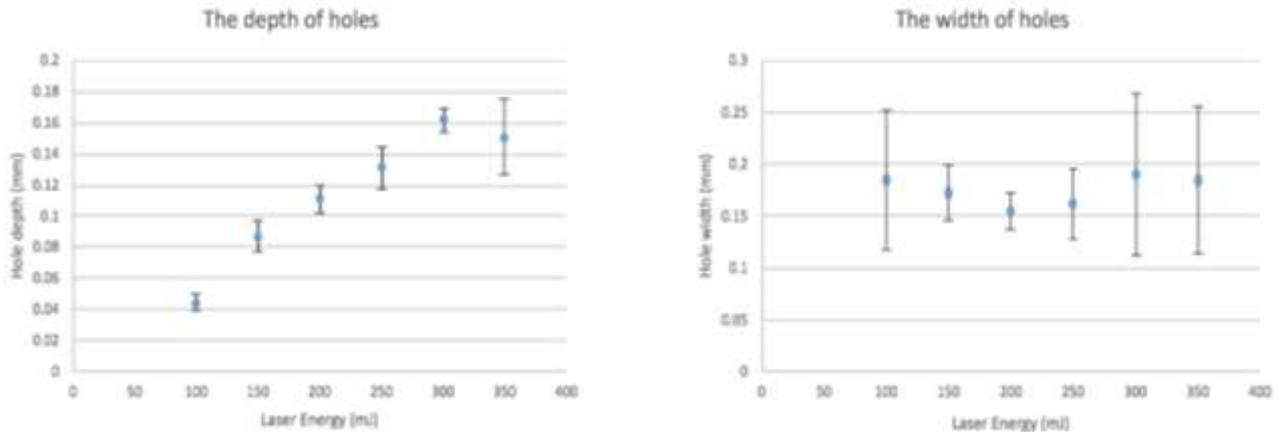


Figure 5. The depth and width of hole changes on the porcine skin specimens treated by different laser energy

#### 4. DISCUSSION AND CONCLUSION

As a high-resolution imaging technology, OCT system can produce cross-sectional images in micrometer level, which can lead us to explore the morphology performance after AFL treatment. From the result part, it indicated that hole depth would raise with the increase of laser treatment energy from 100 mJ to 350 mJ at the increase interval 50 mJ. Although the hole depth in the specimens with 350 mJ laser treatment seldom continued growing and even dropped back to some extent, the increasing trend of the depth of the hole generated by laser beam is dominant. For the shrinking occurring on 350 mJ, one hypothesis proposed was that the depth of hole generated by laser beam stop increasing after laser energy reach around 350mJ applied on the porcine skin sample. Therefore, one further study is necessary to study the morphology of porcine skin sample treated by the laser with 350mJ and higher energy.

From the analysis based on Figure 4 and Figure 5, it can predict that the increase of laser beam energy has no influence over the width of holes after AFL treatment. The main reason might be the principle of the laser beam energy (power x time), which means that the change of the energy was only determined by the treated time (the power is fixed for the laser machine applied in this study), while the diameter of the laser beam kept same under different energy output. The depth of holes can be affected through modifying the treat time but the diameter of the holes was only determined by the shape of the laser beam.

Scattering near the irradiated region might be another factor impacted by the laser treatment. Higher scattering intensity prevented light penetrated into the deeper layers, thus causing the shadow area beneath the holes. The ablative treatment might alter tissue properties, especially enhancing light reflection index.

In conclusion, this paper presented a novel method to image the morphology of porcine skin samples after the ablative laser treatment. Holes would leave on the skin surface after the laser irradiation. The depth of holes increased while the laser energy is getting larger, namely increasing the irradiated time, but the diameter of the holes is independent on laser energy change. In this experiment, OCT system reveals the high-resolution, noninvasive and real-time properties, and reveal the potential and build the foundation to explore the how the ablative fractional laser treatment has an influence over the human skin specimens under different energy irradiation.

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