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ORIGINAL ARTICLE

# *In vivo* characterization of healthy human skin with a novel, non-invasive imaging technique: line-field confocal optical coherence tomography

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## Abstract

**Background** Line-field confocal optical coherence tomography (LC-OCT) is a non-invasive optical technique recently developed for skin examination *in vivo*. It provides real-time, high-resolution vertical images with an isotropic resolution of ~1 μm and a penetration depth of ~500 μm.

**Objectives** Study goals were to qualitatively/quantitatively characterize healthy skin at different body sites using LC-OCT.

**Methods** The skin of young healthy volunteers was imaged with a handheld LC-OCT imaging device. Seven body sites (back of the hand, forehead, cheek, nose, chest, forearm and back) were investigated. An independent qualitative [cutaneous structures' description; visibility of keratinocytes' nuclei and dermal–epidermal junction (DEJ)] and quantitative [*stratum corneum* (SC)/epidermal thicknesses; height of dermal papillae] assessment of the LC-OCT images was performed.

**Results** A total of 88 LC-OCT images were collected from 29 participants (20 females; nine males; mean age 25.9 years). Keratinocytes' nuclei and DEJ were visible in the totality of images. The different layers of the epidermis and the remaining cutaneous structures/findings were visualized. Body sites-related variability was detected for SC/epidermal thicknesses and height of dermal papillae. Inter-observer agreement was excellent (SC thickness), good-to-excellent (epidermal thickness) and moderate-to-good (papillae).

**Conclusions** Line-field confocal-OCT provides non-invasive, real-time imaging of the skin *in vivo* with deep penetration and high resolution, enabling the visualization of single cells. The histology-like vertical view provides an easy way to recognize/measure different cutaneous structures/findings. LC-OCT appears as a promising technique for the examination of physiological/pathological skin.

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## Conflicts of interest

Arnaud Dubois is the inventor of the patent on the LC-OCT technology. He is co-founder of the company DAMAE Medical.

## Funding sources

None.

## Introduction

Several optical techniques have been developed over the last decades in order to image the skin *in vivo*, in real time and non-invasively.[1] Among them reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) are the most used techniques in clinical practice. RCM provides only en-face sectional images of the skin (contrary to conventional histopathology), with an excellent optical resolution of ~1 µm but a penetration depth limited to ~250 µm.[2] Conventional OCT allows the observation of the skin in a vertical plane up to a depth of ~1 mm but with a limited resolution of ~7.5 µm,[3] not sufficient to discern single cells and to distinguish the different layers of the epidermis in detail.[4] High-definition OCT (HD-OCT) was subsequently developed to achieve a higher isotropic resolution of ~3 µm with a penetration depth of ~570 µm.[5] Unfortunately, its commercialization was cancelled by the manufacturers and the technology is not available at the moment.

Recently, line-field confocal OCT (LC-OCT) was developed to meet the need for an *in vivo* non-invasive imaging device with deep penetration and high resolution dedicated to dermatology. This imaging modality combines the physical principles of time-domain OCT and RCM with a penetration up to a depth of ~500 µm and with an isotropic resolution of ~1 µm.[6] By combining the advantages of OCT (high penetration) and RCM (high resolution), this tool seems to be particularly suitable for the examination of both healthy and pathological skin.

The objective of this study was to evaluate the potential of LC-OCT for skin imaging. To that aim, we performed qualitative and quantitative analyses of LC-OCT skin images taken from young healthy volunteers.

## Materials and methods

### Population

The study was performed on healthy volunteers attending the Dermatology Department of Saint-Etienne University Hospital. Inclusion criteria were age <40 years and willingness to participate in the study. Patients allergic to paraffin oil were excluded. The study received approval by the local ethical committee (ID-RCB: 2018-A02012-53).

### LC-OCT device

Images were acquired with a CE-marked prototype of LC-OCT (DAMAE Medical, Paris, France) by one investigator (JLP) expert in skin imaging. The device is composed of a handheld probe connected to a mobile chart embedding a central unit and a display.[6] LC-OCT is a time-domain OCT technique using line illumination of the skin and line detection of the signal instead of point scanning and point detection as in conventional OCT. In this study, we employed a LC-OCT device that produces B-scans (vertically-oriented sectional images) without lateral scanning of a light beam. The parallel acquisition using a line camera allows a reduction in depth scanning speed without increasing the image acquisition time. Dynamic focusing is therefore highly facilitated in LC-OCT compared to point-scanning time-domain OCT. A high numerical aperture objective can be employed to image with high lateral resolution. B-scans of skin were obtained in real time at 10 frames per second with an isotropic resolution of ~1 µm, down to a depth of 500 µm and over a lateral field-of-view of 1.2 mm. The time needed to acquire a LC-OCT image once the probe was in contact with the skin was less than one second, *that is* the time necessary to click the acquisition button on the handheld probe. The time needed to acquire a LC-OCT video corresponded to the duration of the video itself, which was arbitrarily set to 10 s.

### Image acquisition protocol

The glass window at the end of the LC-OCT probe served as a mechanical interface between the objective lens of the probe and the participants' skin. A drop of paraffin oil was placed between the skin and the glass window to ensure refractive index matching and diminish the specular reflection on the skin surface and the glass window. In addition, this window gently flattens and stabilizes the area to be imaged. Live images were directly visualized on the screen, while the operator smoothly moved the tip of the probe over the skin. The procedure was repeated on several body locations. One image per body site was saved for quantitative measurements. Additional images and videos were acquired when a structure of interest was visualized.

### Qualitative image evaluation

Images were studied by all investigators in order to describe the main epidermal and dermal structures/findings [*stratum corneum* (SC), single keratinocytes, DEJ, dermal papillae, collagen/elastic fibres, blood vessels, hair follicles, sebaceous glands, *Demodex folliculorum* and comedones]. Moreover, each image was evaluated independently by three dermatologists (JM, LT and MM) to rank the visibility of the nuclei of keratinocytes and of the DEJ, using the following qualitative scale (3: good; 2: partial; 1: no visibility). Any disagreement was solved by consensus among them. To assess the correlation between LC-OCT and histology, we took LC-OCT additional images of the healthy skin adjacent to a basal cell carcinoma (BCC) before its surgical removal in a 69-year-old female patient.

### Quantitative image evaluation

For each LC-OCT study image, three regions of interest (ROI) were established by evenly positioning three 20-µm wide rectangles within the 1.2 mm field-of-view (Fig. 1a). The three evaluators were then asked to measure the thickness of the SC and the whole epidermis (including the SC) within these three ROI (Fig. 1b,c). The rationale behind choosing three ROI within each study image was to obtain measurements independent from the

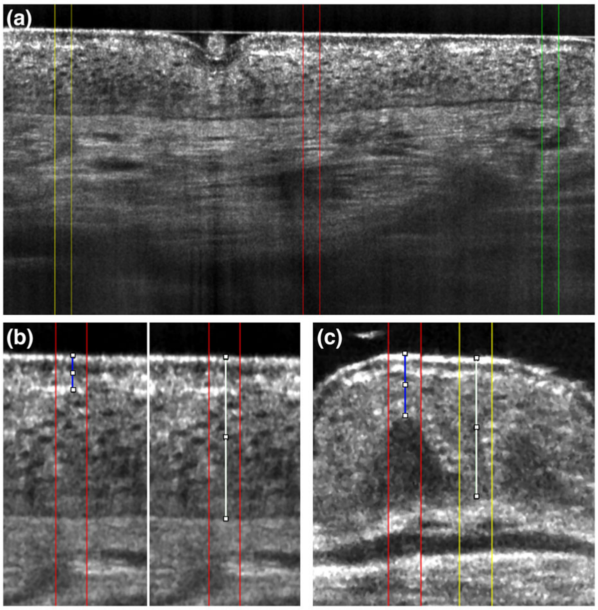


**Figure 1** Quantitative image evaluation. (a) For each line-field confocal optical coherence tomography (LC-OCT) study image, three regions of interest (ROI) were established by evenly positioning three 20-μm wide rectangles (yellow, red, and green) within the 1.2 mm field-of-view. (b) Images were displayed with a 150% zoom and the evaluators were asked to carefully draw, within the ROI, a first vertical segment (blue) going from the skin surface to the end of the SC and a second one (white) going from the skin surface to the dermal–epidermal junction. (c) In the presence of rete ridges, the thickness of the epidermis was calculated as the average of the length of the segment connecting the skin surface to the top of the papilla (blue) and the segment connecting the skin surface to the bottom of the papilla (white). Measurements were taken by using the ImageJ line measurement tool. The LC-OCT images were taken from the back of the hand of a phototype III, 30-year-old female participant (a, b) and from the back of a phototype II, 22-year-old male participant (c).

natural variation of the skin thickness. Measurements were performed with ImageJ (NIH, version 1.51j8, Bethesda, MD).

### Statistical analysis

Continuous variables were reported as means with standard deviations and inter-individual variation (expressed as percentages). Means were compared with Student's *t*-test. Inter-observer agreement was assessed by the intra-class correlation coefficient (ICC), using two-way random-effects models (ICC $< 0.5$: poor agreement; $0.5 \leq$ ICC $< 0.75$: moderate agreement; $0.75 \leq$ ICC $< 0.9$: good agreement; ICC $> 0.9$: excellent agreement). The ICC was presented along its 95% confidence intervals (CI). The statistical analysis was carried out with the R statistical software v3.6.0, IRR package v3.6.1.[7]

## Results

### Study population and images

A total of 29 individuals (20 females and nine males; mean age 25.9 ± 4.8 years) participated in the study. Two body sites (back of the hand and middle part of the forehead) were imaged in 19 volunteers (11 females and eight males – mean age 27.1 ± 5.2 years – phototype II: 4; III: 14; V: 1). Five additional body sites (cheek, tip of the nose, upper chest, posterior forearm, and back) were imaged in 10 healthy volunteers (nine females and one male – mean age 23.8 ± 2.6 years – phototype II: 8; III: 1; IV: 1). Therefore, a total of 88 LC-OCT images were collected.

### Qualitative analysis

*Skin structures and findings visible with LC-OCT* All investigators detected the main epidermal/dermal structures (Fig. 2). The SC was hyper-reflective on LC-OCT and clearly visible. A hyper-reflective linear signal distinguished the SC from the underlying *stratum granulosum*. Single keratinocytes under the SC were distinguishable due to their hypo-reflective nuclei, which were stretched in the *stratum granulosum* and roundish in deeper layers. Contours of single keratinocytes could not be defined. The dermis displayed an overall hyper-reflective signal due to the presence of collagen/elastic fibres, appearing as bright, wavy, linear structures. The DEJ appeared as a hypo-reflective line clearly separating the epidermis from the dermis: its shape could be wavy (rete ridges) or flat (flattening of the rete ridges) according to the presence or, respectively, absence of dermal papillae. The representation of the dermal papillae varied across different body sites and individuals (Fig. 3). The DEJ had good visibility and partial visibility in 68% and 32% of cases, respectively; the visibility of keratinocytes' nuclei was good in 59% of cases and partial in 41% of cases. Darker phototypes (IV–V) could be clearly differentiated from fairer phototypes (II–III) due to a higher hyper-reflectivity of the epidermal basal layer in the former (Fig. 4a). Hair follicles were characterized by a homogeneously hypo-reflective infundibulum, a homogeneously medium-reflective hair shaft and a hair follicle epithelium composed of cells similar to epidermal keratinocytes. Hair follicles had different shape according to their orientation in each particular image: thin and elongated structures crossing the epidermis and reaching the skin surface or roundish dermal structures (Fig. 4b, Video S1, Supporting Information). In the dermis, sebaceous glands appeared as large hypo-reflective roundish structures adjacent to the hair follicle (Fig. 4b) and blood vessels were clearly visible as well-defined, hypo-reflective linear structures of various shape/size (Fig. 4c). The presence of *D. folliculorum* was detected as an intensely hyper-reflective elongated structure within the hair follicle (Fig. 4d).

 

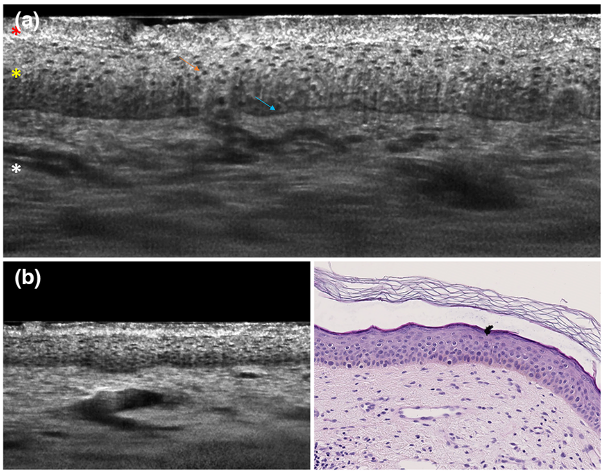


**Figure 2** Main epidermal and dermal structures visible at Line-field confocal optical coherence tomography (LC-OCT) and histopathology. (a) At LC-OCT examination, all different layers of the skin are visible. The *stratum corneum* (red star) appears as a hyper-reflective band. The epidermis (yellow star) features keratinocytes with hypo-reflective nuclei (orange arrow) and is composed of its different layers: the *stratum granulosum* with stretched nuclei, the *stratum spinosum* with smaller, roundish nuclei and the *stratum basale*, immediately adjacent to the dermal–epidermal junction. The dermal–epidermal junction (blue arrow) appears as a hypo-reflective line clearly separating the epidermis from the dermis. The dermis (white star) contains hyper-reflective (collagen and elastic fibres) and hypo-reflective (vessels) linear structures. The image was taken from the back of the hand of a phototype II, 35-year-old female participant. (b) LC-OCT/histopathology correlation. The image was taken from the chest of a phototype III, 69-year-old female participant on the healthy skin adjacent to a basal cell carcinoma before its surgical removal.

Comedones appeared as large keratinizing structures dilating the infundibulum (Fig. 4d, Video S2, Supporting Information).

*Histological correlation* High level of similarity between LC-OCT and histology was observed in the healthy skin adjacent to a BCC. Interestingly, the SC was attached to the epidermis on the LC-OCT image and separated from it in the histological image (Fig. 2).

### Quantitative analyses

*Skin variations across different body sites and individuals* The seven imaged body sites differed as for the SC/epidermal thickness and the presence/dimension of dermal papillae (Fig. 3). The SC was much thicker on the back of the hand with a mean thickness of 29.5 μm compared to the other body sites for which the mean SC ranged from 9.0 μm (cheek) to 12.7 μm (forearm; $P < 0.001$; Table 1). Inter-individual variation of the SC thickness ranged from 13% (cheek) to 25% (forearm). The epidermal thickness showed significant variation on the different body sites, with almost a ratio of two between the largest (back of hand, 98.9 μm) and the thinnest (chest, 54.3 μm; $P < 0.001$). Papillae were most prominent on chest and back. Cheek and nose did not show any papillae on any volunteer. The cheek presented a completely flat DEJ, while the nose presented in some cases an undulating silhouette of the DEJ (Fig. 3b). High inter-individual variation was found for forehead, forearm and back of hand: 5/10 (50%) participants imaged on the forearm and 9/19 (47.4%) participants imaged on the forehead and back of hand presented clear papillae while the rest of them had a flat DEJ.

The inter-observer agreement was excellent for SC thickness, good-to-excellent for epidermal thickness and moderate-to-good for dermal papillae height (Table 1).

## Discussion

Our qualitative analysis showed that LC-OCT is able to visualize the different cutaneous structures with cellular level definition. Indeed, keratinocytes were identified in the totality of the images, thanks to their hypo-reflective nuclei. The dimension and shape of the nuclei as well as the vertical imaging allowed differentiating the epidermal layers. The fact that in 41% of the images the keratinocytes' nuclei were only partially visible is probably due to the fact that (i) the device used here was an early prototype with variable image quality depending on factors such as patient's compliance, amount of immersion oil used and presence of make-up residuals; (ii) at the time of the study the evaluators had limited experience with the device. Further studies using more advanced LC-OCT devices (soon available) with higher degree of experience should provide even better results in this regard. Similarly, the DEJ was detected in all study cases as a hypo-reflective line clearly separating the epidermis from the dermis. Its visibility was deemed good in the majority of cases, especially in dark-skinned participants due to the hyper-reflectivity of the basal layer of the epidermis, as previously shown with HD-OCT.[5] The main remaining cutaneous structures were also visible, including dermis, blood vessels, hair follicles and sebaceous glands. Additionally, comedones and *D. folliculorum* were discernible within the hair follicles. Melanocytes, Langerhans/dendritic cells and dermal inflammatory cells were not evaluated in this study, as they cannot be clearly visualized by LC-OCT in normal skin. Conversely, LC-OCT is able to highlight these cellular types in benign/malignant skin tumours as well as in inflammatory cutaneous diseases (unpublished data).

The high level of similarity between the histopathological section and the corresponding LC-OCT image of the skin adjacent to a BCC prior to its excision was reassuring, regardless of whether perilesional skin is indeed to be considered 'normal' or

 

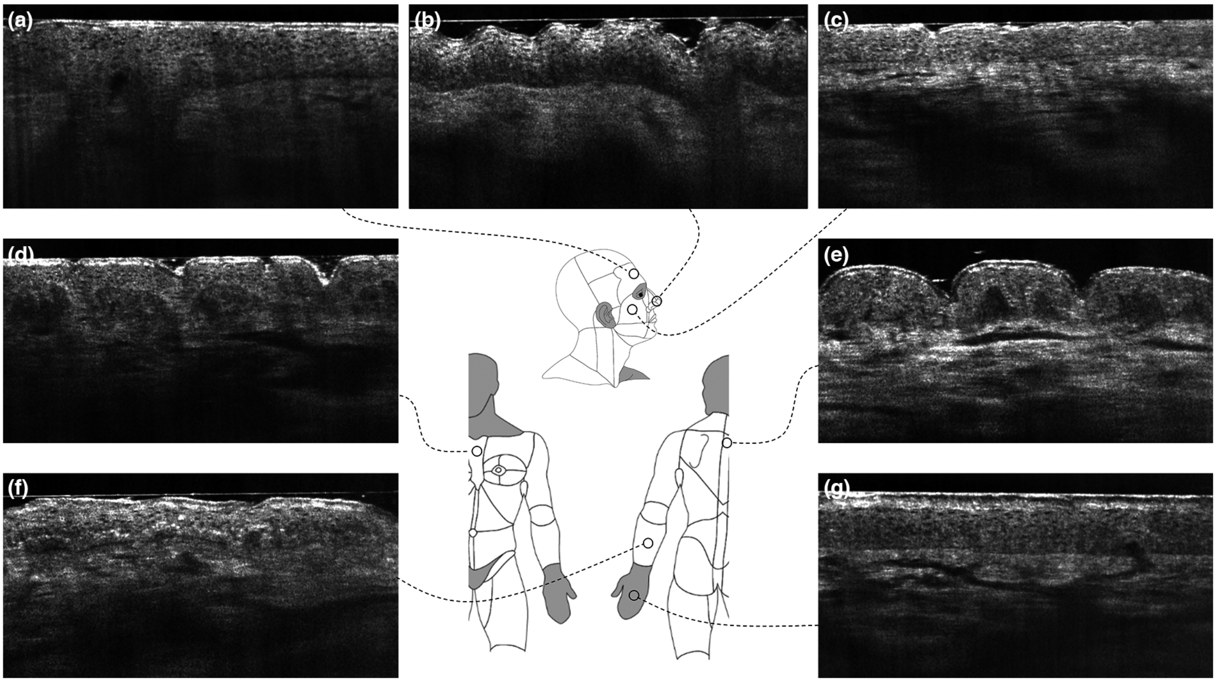


**Figure 3** Qualitative and quantitative variability of Line-field confocal optical coherence tomography findings across different body sites. (a) Forehead: thin *stratum corneum*, thick epidermis, flat dermal–epidermal junction (DEJ; phototype II, 30-year-old female). (b) Nose: thin *stratum corneum*, thick epidermis, slightly wavy DEJ (phototype II, 22-year-old female). (c) Cheek: thin *stratum corneum*, thin epidermis, flat DEJ (phototype III, 27-year-old female). (d) Chest: thin *stratum corneum*, thin epidermis, wavy DEJ (rete ridges) (phototype III, 27-year-old female). (e) Back: thin *stratum corneum*, thin epidermis, wavy DEJ (rete ridges) (phototype II, 22-year-old male). (f) Forearm: thin *stratum corneum*, thick epidermis, slightly wavy DEJ (phototype IV, 27-year-old female). (g) Hand: thick *stratum corneum*, thick epidermis, flat DEJ (phototype III, 25-year-old female).

not. The fact that the SC was attached to the epidermis in the LC-OCT image and separated from it in the histological image might be explained by both the processing of the histopathological specimen and the pressure applied on the skin by the LC-OCT probe. Further studies are needed to systematically assess the correlation of LC-OCT with histopathology. In this regard, a recent preliminary investigation showed promising results.[8]

The quantitative analysis showed significant body site-dependent variability of SC/epidermal thickness and presence of the rete ridges. The highest values of SC/epidermal thickness were found on back of hand, forearm, forehead and nose; the most prominent dermal papillae were detected on back and chest. Inter-individual variability was observed: the forearm displayed the highest variations of SC/epidermal thickness; forehead, forearm and back of hand showed the highest variations in terms of rete ridges, with half of the participants clearly presenting dermal papillae and half having a flat DEJ. A notable result of our study was that the measurements were reproducible among the three observers, especially for the SC/epidermal thicknesses. Indeed, they were fairly easy to measure due to the vertical representation and the different refractivity of the SC as compared to the remaining epidermis. The fact that the visibility of the DEJ was partial in 32% of the cases is likely to explain the less impressive reproducibility of the height of the dermal papillae.

The values found for the thickness of the SC in this study are in line with previous investigations using RCM.[9] Conversely, the values found for the thickness of the whole epidermis in this study are superior to RCM[10] and inferior to histology[4,11] and OCT.[3,4] Moreover, epidermal thickness showed more variation across different body sites in this study than in previous assessments with RCM.[10] The differences with RCM may be due to the en-face view being its only available imaging modality, which implies a certain lack of global vision ultimately leading to difficulties in identifying the DEJ level.[9,10] The differences with OCT are probably due to its lower resolution that does not permit to clearly identify the DEJ and the SC.[3,4,12,13] The differences with histology might putatively lie with the fact that this *ex vivo* technique is dependent upon fixation agents that may cause swelling, retraction and hardening of the examined tissue: in this scenario, one could speculate that, although universally considered as the gold standard of tissue quantification, histology

 

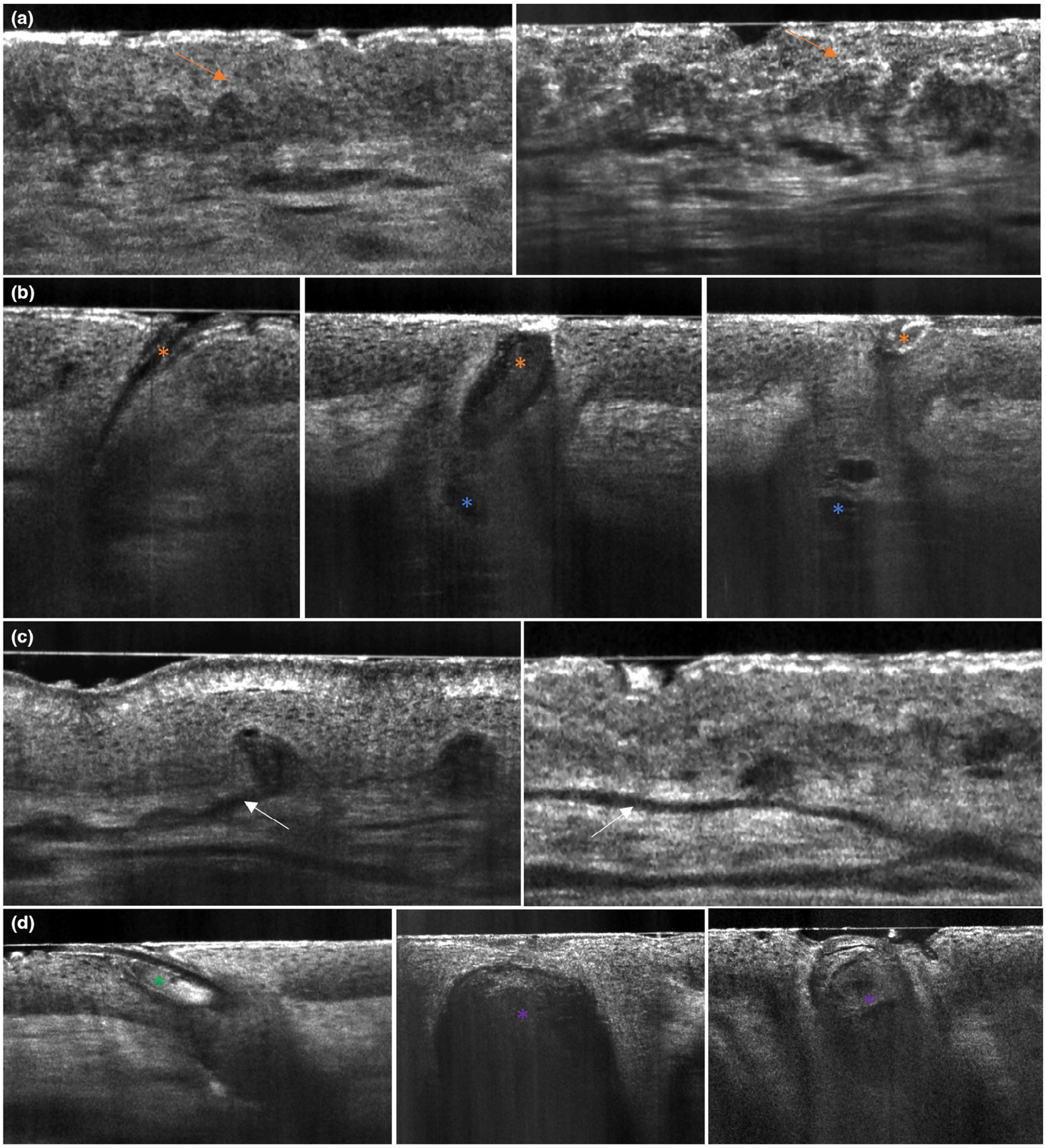


**Figure 4** Additional skin structures and findings visible with Line-field confocal optical coherence tomography. (a) Different reflectivity of the basal layer of the epidermis (orange arrows) in paler phototypes (left image; hypo-reflective basal layer; phototype II, 26-year-old female, back) as compared to darker phototypes (right image; hyper-reflective basal layer; phototype IV, 27-year-old female, back). (b) Hair follicles and sebaceous glands. Hair follicles (orange stars) present different shape according to their orientation within the image: thin and elongated structures crossing the epidermis and reaching the skin surface (left) or roundish dermal structures (middle and right). Sebaceous glands appear as large hypo-reflective roundish structures adjacent to the hair follicle (blue stars). Phototype III, 27-year-old female, forehead. (c) Blood vessels (white arrows) are clearly visible as well-defined, hypo-reflective linear structures of various shape and size: capillaries looping inside the papillae (left; phototype II, 22-year-old male, back of the hand) and long horizontal vessels within the superficial dermis (right; phototype II, 22-year-old male, chest). (d) *Demodex folliculorum* (green star) appears as a hyper-reflective, elongated structure within the hair follicle (left; phototype II, 22-year-old female, cheek). Comedones (purple stars) appear as large keratinizing structures dilating the infundibulum of the hair follicle (middle and right; phototype II, 22-year-old male, nose).

**Table 1** Quantitative evaluation of the skin at different body sites by means of line-field confocal optical coherence tomography (LC-OCT) images

| Body site | Stratum corneum thickness (%) | Epidermal thickness (%) | Height of dermal papillae (%) |
|---|---|---|---|
| Forehead | 11.6 ± 1.7 (15) | 81.1 ± 12.9 (16) | 18.3 ± 21.5 (117) |
| Nose | 10.4 ± 1.4 (14) | 84.4 ± 15 (18) | — |
| Cheek | 9 ± 1.1 (13) | 58.7 ± 9.7 (16) | — |
| Chest | 9.1 ± 1.2 (14) | 54.3 ± 5.9 (11) | 22.6 ± 12.9 (57) |
| Back | 9.5 ± 1.3 (14) | 59.9 ± 4.5 (8) | 27.5 ± 7.2 (26) |
| Forearm (posterior) | 12.7 ± 3.2 (25) | 70.7 ± 12.8 (18) | 7 ± 10.1 (144) |
| Hand (back) | 29.5 ± 5.7 (19) | 98.9 ± 15.6 (16) | 18.7 ± 22.8 (122) |
| **Inter-observer agreement (ICC; 95% CI)** | **0.95 (0.91–0.97)** | **0.87 (0.82–0.91)** | **0.72 (0.56–0.83)** |

Mean thickness ± standard deviation (inter-individual variation) is presented in each box, unless otherwise stated.
Measures are expressed in μm.
For each body site, the average thickness among all participants is reported. The inter-participant thickness variation is expressed both as standard deviation and percentage.

might not truthfully account for the real thickness of the cutaneous layers. Additional studies are needed to better clarify this issue.

This study had several limitations. Not all anatomic locations were assessed and different body sites were studied in the two participants groups; one group had an inhomogeneous gender distribution, and overall the sample size and the phototypes' representation were not extensive. However, the aim of the study was to provide a preliminary, general description of the main features of normal skin; future investigations will explore more systematically the entire integumentary system within all skin types. Similarly, additional studies are required to investigate different age groups than those included in the present investigation, as the focus here was to describe healthy skin in young (<40 years old) individuals. Only one illustrative example of histological correlation was provided in a subject not included in the study, because performing invasive surgical procedures was deemed unethical in this non-interventional study on healthy volunteers.

Line-field confocal-OCT offers an *in vivo*, non-invasive examination in the vertical plane with high resolution, which can be particularly suitable for physiological studies of the skin. Characterizing various skin structures at different body sites helps to better understand the normal anatomic variability of the skin and could therefore improve the diagnostic accuracy for different skin conditions.

It has been suggested that novices to RCM need a long training period before being able to interpret the images.[14] LC-OCT images seem easier to interpret because of their vertical orientation, similar to histology. Only a small amount of training may be required to use this technique by professionals already trained in pathology. This potential advantage of LC-OCT over RCM needs to be investigated in future studies.

Some interesting clinical applications of LC-OCT are to be expected, including diagnosis of skin cancers and inflammatory diseases; biopsy guidance; identification of tumours' surgical margins, and follow-up of topical/systemic treatments. A LC-OCT device capable of producing three-dimensional (3D) images (both in the vertical and horizontal plane), currently under development, will be used for further studies.[15] This device will help to better understand not only the 3D structure of the skin, but also the distribution patterns of blood vessels and melanin thanks to 3D modelling. Another prospect under development is the automatic DEJ detection and epidermal thickness measurement.

In conclusion, we presented the first study describing the LC-OCT features of normal skin. Based on these preliminary findings, LC-OCT seems to be very promising for dermatology with a wide field of possible applications in the physiological/pathological spectrum of the skin.

## Acknowledgements

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Video S1.** Hair follicles. Hair follicles can have different shape according to their orientation in each particular image: thin and elongated structures crossing the epidermis and reaching the skin surface or roundish dermal structures. Video taken from the nose of a phototype II, 22 year-old female participant.

**Video S2.** Comedo. Comedones appear as large keratinizing structures dilating the infundibulum. Video taken from the nose of phototype II, 21 year-old female participant.