Clinical and Laboratory Investigations

Dermatology

Dermatology 740
DOI: 10.1159/000XXXXXX

Received: June 26, 2007
Accepted: September 13, 2007
Published online: ■■■

Morphology and Epidermal Thickness of Normal Skin Imaged by Optical Coherence Tomography

Mette Mogensen[a] Hanan A. Morsy[a] Lars Thrane[b] Gregor B.E. Jemec[a]

[a]Department of Dermatology, Roskilde Hospital, University of Copenhagen, and [b]Optics and Plasma Research Department, Risø National Laboratory, Technical University of Denmark, Roskilde, Denmark

Key Words
Optical coherence tomography, polarization-sensitive · Epidermal thickness, normal skin · Diagnostic imaging

Abstract
Background: Optical coherence tomography (OCT) is an optical imaging technology with a potential in the non-invasive diagnosis of skin cancer. To identify skin pathologies using OCT, it is of prime importance to establish baseline morphological features of normal skin. ***Aims:*** The aim of this study is to describe normal skin morphology using OCT and polarization-sensitive OCT (PS-OCT), which is a way of representing birefringent tissue such as collagen in OCT images. Anatomical locations in 20 healthy volunteers were imaged, and epidermal thickness (ET) was measured and compared to age, gender and skin colour. ***Methods:*** OCT imaging is based on infrared light reflection/backscatter from tissue. PS-OCT detects birefringence of tissue. Imaging was performed in 12 skin regions. ET was calculated from the OCT images. ***Results:*** Normal skin has a layered structure. Layering is less pronounced in adults. In glabrous skin the stratum corneum is visible. Children had larger ET ($p < 0.0001$). Age had a negative correlation with ET ($p < 0.05$). No gender- or skin-type-related differences in ET were found. ***Conclusion:*** This study contributes to understanding OCT and PS-OCT images of normal skin and indicates that OCT can be used for both the qualitative and quantitative assessment of skin.

Introduction

Several new imaging techniques are being studied in dermatology, especially in the field of skin cancer [1, 2]. These techniques offer examination of the skin with a greater level of magnification than previously and add functional information. A variety of dermatological disorders have been investigated using various non-invasive imaging techniques in vivo: ultrasound [3, 4], dermoscopy [5, 6], confocal laser scanning microscopy [2, 7], magnetic resonance imaging [8, 9], multiphoton microscopy [10], terahertz imaging [11] and optical coherence tomography (OCT) [12–14]. Before these methods can be clinically integrated there is a need for structured testing akin to that known from therapeutic drug development. Diagnostic research involves 4 phases [15]. The first phase involves studies of normal skin and determination of the normal range of values for a diagnostic test through observational studies in healthy people. An extensive material of normal skin has been studied by ultrasound, but

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–8665/08/0000–0000$24.50/0

Accessible online at:
www.karger.com/drm

Mette Mogensen, MD
Department of Dermatology
University of Copenhagen, Roskilde Hospital
DK–4000 Roskilde (Denmark)
Tel. +45 4732 2116, Fax +45 4732 2128, E-Mail rsmmog@ra.dk

few studies with other imaging technologies have been concerned with the morphology of the normal skin. Four previous OCT studies have been mainly concerned with the description of the normal skin: one investigated the changes in normal skin induced by tape stripping, intracutaneous histamine, various ointments and ultraviolet radiation [16]. The second was more concerned with the measurement of epidermal thickness (ET) than the description of variance in morphological features per se [17]. In the third, thickness of the stratum corneum (SC) was measured in all fingers of 87 healthy volunteers and 18 people with diabetes [18]. The SC was thinner at the sides of the tactile elevations than at the centre, and it was thinner in women than in men. Lastly a study applied both confocal laser scanning microscopy and OCT and found a significant decrease in the maximum thickness of the epidermis and flattening of the dermo-epidermal junction with age [19].

OCT has shown potential in the characterization and diagnosis of a variety of dermatological disorders [1, 13]. The ET can be quantified easily, which may be a relevant applicability of OCT [17, 20]. It is not straightforward to measure ET in histological sections. The main problem for in vitro measurements is that during routine histological formalin-paraffin preparation the excised skin undergoes distortion and shrinkage and/or swelling. This may be overcome by cryopreparation that prevents tissue crystallization, conserves tissue structures better and has a higher accuracy in estimation of ET [21]. OCT has also been suggested as a method for measuring skin tumour thickness [22]. Other characteristic OCT features have been studied following ultraviolet radiation [23], in basal cell carcinomas [12, 24, 25], in actinic keratosis [26], systemic lupus erythematosus [27], patch test reactions [28], bullous diseases [14], psoriasis [29], tattoos [30], scabies [31] and cutaneous larva migrans [32].

Recognition of the typical appearance of the normal skin in different body regions is of vital importance in dermatological research, as knowledge of the OCT skin morphology under the effect of age, gender, location and skin type is of prime value to understand disease processes and therapeutic effects of treatments. Qualitative morphological analysis of normal skin OCT is sparse, although it is stated that OCT may be an effective tool to visualize skin structures such as the epidermis, papillary dermis and skin appendages like hair follicles and eccrine ducts [33]. Our objective is to describe the qualitative morphology of OCT images of normal skin in various locations of the body.

Polarization-sensitive OCT (PS-OCT) is a way of representing birefringent tissue such as collagen in OCT images, and it was applied to add functional information to OCT images in this study. Furthermore, we wanted to do ET measurements with our OCT system to relate our study to the work done earlier with OCT [17]. However, our main scope is to study the qualitative potential of OCT in describing samples from normal skin.

Materials and Methods

Twenty healthy volunteers were enrolled in accordance to Helsinki II, and the study was approved by the Local Ethics Committee (ref. No. 2005-1-41). Volunteers were recruited and OCT imaged in the period from December 2006 to March 2007. History of any dermatological disorder was one of the exclusion criteria. OCT imaging was done in 12 predetermined anatomical sites: forehead, ear lobe, nose, cheek, chin, back of the neck, chest, hand (both sides), arm (both sides) and calf.

The OCT system used in this study was developed at Risoe National Laboratory, Denmark. The hand-held OCT probe is fibre based and easy to handle. The probe is applied directly to the skin, using ultrasound gel to improve image quality [34]. The scanning lasts few seconds.

OCT works in analogy to ultrasound. It measures the intensity of reflection/backscatter of infrared light (instead of acoustical waves) from the skin. Because the velocity of light is extremely high, optical echoes cannot be measured directly. OCT is therefore based on low-coherence interferometry that correlates reflected/backscattered light from tissue with light that has travelled a known reference path. The image data are displayed by assigning grey scales to each reflection according to the measured signal strength [35, 36]. Characteristics other than the intensity of backscattered/reflected light can also be imaged by OCT [25, 37]. Some tissues such as muscle and collagen are birefringent, and in PS-OCT the characteristic birefringence of tissue is imaged. In our OCT system, PS-OCT images are recorded in parallel with standard OCT images. The light source of the OCT system is a superluminescent diode with a centre wavelength of 1,318 nm, and a full width at half maximum bandwidth of 66 nm. Axial resolution is 8 μm and lateral resolution 24 μm. The lateral resolution is in the lower range, but it was chosen in order to obtain a high penetration depth due to the lateral resolution/penetration depth trade-off. The maximum penetration depth in skin is around 2 mm dependent on the backscattering/scattering properties of the skin. Accordingly in highly reflective, hyperkeratotic skin, penetration is low and as high as 2.2 mm in bullous skin due to the low scattering characteristics of the fluid.

ET Measurements

An integrated software program for determination of distances in OCT images (B scans) was developed by Risoe National Laboratory and used in this study. The epidermis was delineated manually on the computer screen, and the mean ET was calculated from 5 predefined ET measurements: the central ET of the OCT image and 2 lateral ET measurements on each side at a fixed distance from the centre, similar to the procedure in the study of

Gambichler et al. [17]. All ET measurements in OCT images were made by the same investigator (H.A.M.). Earlier studies have shown a good morphological correlation between OCT images of normal skin and cryopreserved skin imaged by microscopy [20]. Biopsies were therefore omitted as the intention of this study was to describe the well-known regional variation of OCT image morphology and not to compare OCT with skin histology.

Colour

Together with OCT imaging we measured the colour parameters for each person (L*a*b* values) using the tristimulus colorimeter Chroma Meter CR-400M (Minolta, Japan) that quantifies colours as L*a*b* values, with L* representing brightness [38]. Skin colour was measured on the upper palmar aspect of the arm and on the cheek. The colorimetric L* measurements were compared to ET on the cheek and the palmar aspect of the arm. Fitzpatrick skin type was also compared to ET.

Statistics

A Graph Pad Prism 4 software program was used. Two-way ANOVA was used in comparing adult and child ET, as well as gender and skin type differences in ET. The Pearson correlation procedure was used to assess the relationship between quantitative variables for age and ET. A p value <0.05 was considered statistically significant.

Results

Twenty volunteers with a mean age of 34 years (from 6 months to 59 years) were recruited (table 1).

OCT Morphology of Normal Skin

OCT images reflected the well-known layered architecture of the skin, and a demarcation between epidermis and dermis was evident in most of the images. In images with a subtler demarcation, the layers could still be separated. OCT images of all anatomical locations are shown in figure 1. The epidermis was less reflective/backscattering than the dermis. We defined the border between epidermis and dermis as the first increase in OCT image intensity after the entrance signal. In OCT images from the palm, a dark grey band on top of the epidermis represented the SC. OCT images of the calf, lower arm and dorsum of the hand demonstrated a layered structure, but in the dermis, dark elongated light-absorbing or low backscattering structures sized 0.1–0.4 mm in depth and sometimes more than 4 mm long appeared. The epidermis of the dorsum of the hand and back of the neck both had a more rugged surface compared to the other anatomical sites studied. The layering of the skin was less pronounced in adults on the nose, forehead, chin, cheek and chest than elsewhere, which may be due to the presence of sebaceous glands and hair follicles. In some adults we found a downward directed dark pointing structure stretching through the epidermis to the upper dermis. These structures were most often identified in the chin, cheek, nose and dorsal aspect of the lower arm. These structures were not found in the scanned children. In the helix of the ear some dark homogenous elongated, curved and twisted structures sized 0.1–0.2 mm in width and 1–3 mm in length appeared, and these structures were evident in 17 out of 20 helices. In glabrous skin of the palms, the SC was clearly visible in all images.

PS-OCT images of normal skin showed a characteristic structure due to the arrangement of the non-birefringent epidermis and low birefringent papillary dermis opposed to the dermis containing birefringent col-

Table 1. Population data

Person	Age, years	Gender	Fitzpatrick skin type	L* cheek	L* arm
Adults					
1	52	F	3	61.67	67.6
2	30	F	4	60.67	63.65
3	33	F	2	67.25	68.86
4	48	M	2	62.43	69.27
5	55	F	3	61.22	64.28
6	42	M	2	61.87	70.82
7	30	M	3	62.59	69.27
8	40	M	3	63.2	68.07
9	59	M	2	57.58	65.16
10	42	M	3	62.45	68.63
11	49	M	3	60.11	69.96
12	32	M	2	67	69.26
13	39	M	3	62.54	70.16
14	40	F	2	62.03	67.78
15	48	M	2	61.55	67.65
16	29	M	4	57.5	59.06
Mean	41.75			61.98	67.47
SD	9.13			2.51	2.95
Children					
17	0.5	F	4	55.95	61.1
18	3.5	F	5	55.73	59.5
19	2.5	F	2		
20	5.5	M	2		74.04
Mean	3			55.84	64.88
Mean all	34			61.3	67.06
SD	17.99			3.14	3.96

ET was measured with OCT and skin type with a tristimulus colorimeter. The L* values from the tristimulus colorimeter are in the centre columns. F = Female; M = male.

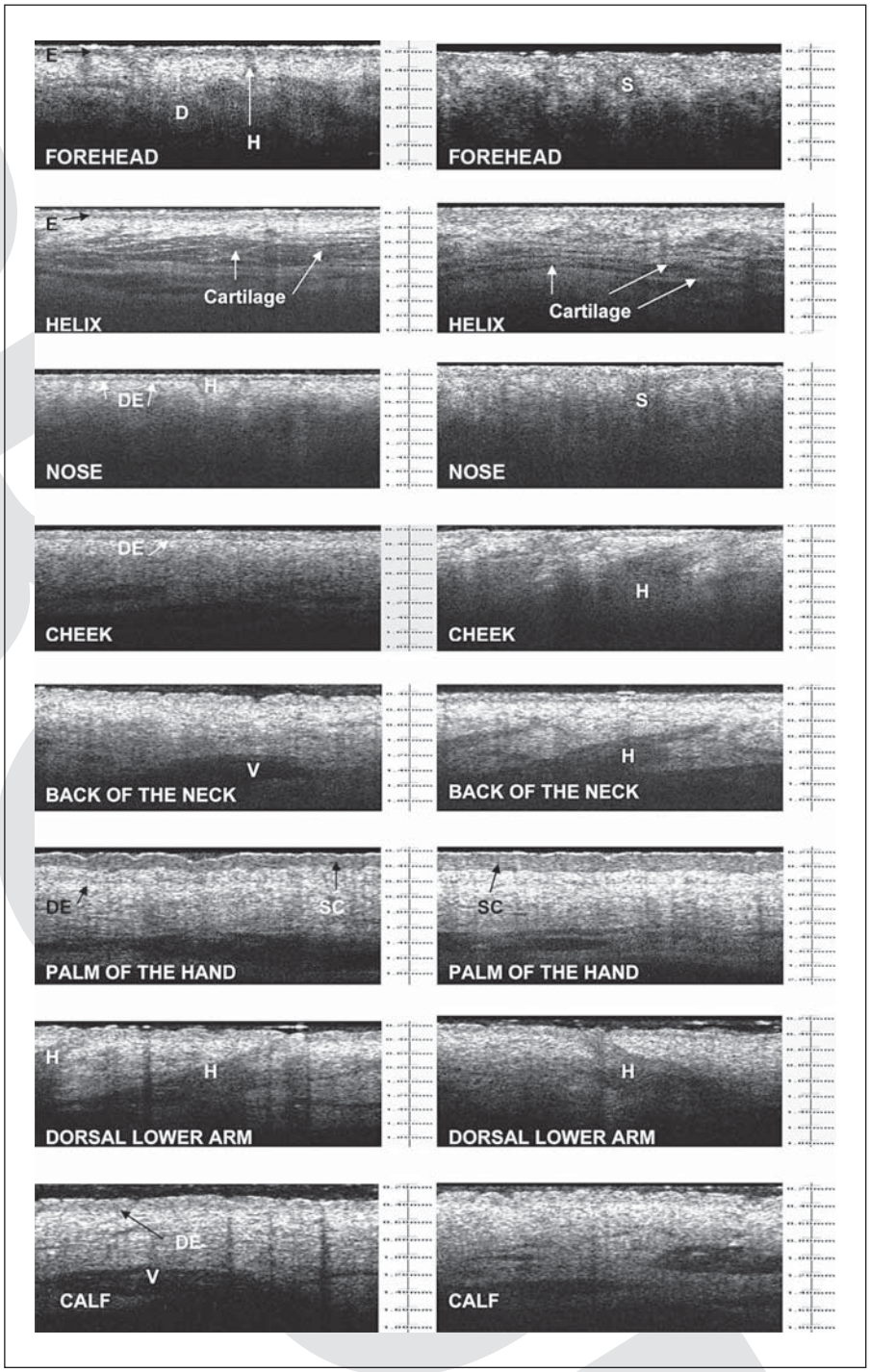

Fig. 1. OCT images from various anatomical locations. These OCT images were selected among the 20 volunteers to represent the typical OCT morphology of the particular region. E = Epidermis; DE = dermo-epidermal junction; D = dermis; H = hair follicle; S = sebaceous gland; V = vessels. Artefacts are marked with arrows.

lagen structures. In all PS-OCT images the epidermis and papillary dermis showed a characteristic homogenous light to dark grey signal, with a maximum depth of 0.2 mm in non-glabrous skin. The underlying reticular dermis showed heterogeneous changes in signal from white to black either in the horizontal or vertical direction. This pattern of changes is seen in all PS-OCT images at the reticular dermis level. The dermo-epidermal junction is not well demarcated in PS-OCT images (fig. 2).

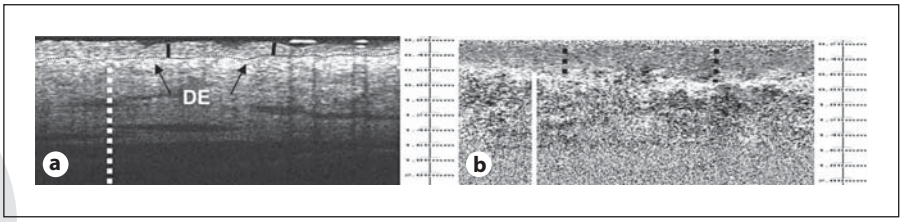

Fig. 2. OCT and PS-OCT image from the palmar antebrachium. **a** OCT image. The dermo-epidermal junction is delineated and marked with DE and characterized by a horizontal change from low intensity to higher intensity. The epidermis is marked by a black bar, the dermis by a white dotted line. **b** Corresponding PS-OCT image. PS-OCT images reflect the epidermis and papillary dermis as a homogeneous upper band, marked with a dotted black bar, and the reticular dermis as a heterogeneous black-and-white lower region of the image, marked with a white bar.

Table 2. Mean ET for various anatomical locations

Anatomical location	Mean ET ± SD, μm
Forehead	88.05 ± 6.46
Ear	85.11 ± 8.27
Nose	86.89 ± 7.68
Cheek	85.03 ± 8.83
Chin	86.77 ± 6.82
Dorsum of hand	88.65 ± 5.67
Palm	113.17 ± 6.82
Palmar antebrachium	87.79 ± 7.34
Dorsal antabrachium	85.96 ± 7.98
Chest	85.52 ± 7.21
Back of the neck	86.65 ± 5.83
Calf	85.87 ± 67.58

Table 3. Relation of ET measured by OCT: differences between adults and children, gender and skin colour

Relation of ET to	Result
Children's and adults' skin	difference between adults and children accounts for 61.70% of the total variance in ET, $p < 0.0001$
Skin colour	
Skin type	difference in skin colour accounts for 0.44% of the total variance in ET, $p = 0.11$
L* value	linear regression shows no correlation between L* values and corresponding ET on cheek and inner arm
Gender	approximately <0.1% of the variation, $p = 0.50$
Anatomical location	difference in anatomical location accounts for 1.31% of the total variance in ET, $p = 0.66$

Data from anatomical locations have a normal distribution if ET of the palm is omitted from the analysis.

p values are assessed by 2-way ANOVA. Children's data are based on very small material.

ET Measurement with OCT

The average ET in various anatomical sites from all persons is shown in table 2. The relation of ET to gender, skin colour and the difference between ET in adults and children is shown in table 3. Gender and skin type do not affect ET. In the adult group the ET significantly differed from that of children, who had a larger ET. The Spearman correlation procedure also showed a statistically significant negative correlation between age and ET in all anatomical locations except from the palm.

Discussion

In vivo OCT and PS-OCT are conceptually different from ordinary light microscopy. Nevertheless, our data indicate that OCT identifies characteristic microscopic features in the skin, which would otherwise only have been obtained from histological sections.

OCT images presented some regional differences: in the helix dark homogeneous wavy structures are consistent with the hyaline cartilage of the ear. In glabrous skin of the palms, the SC is clearly visible in all images. OCT images of the dorsum of the hand, the arm and calf demonstrate dark, elongated dermal structures, which may be blood vessels or lymphatic vessels. In earlier studies these structures have been ascribed to blood vessels; however, if they were all blood vessels, the OCT signal below should be attenuated due to the strong scattering of haemoglobin. The absence of such a signal attenuation suggests

that some of these are lymphatic vessels or areas of less dense collagen tissue. In adults the pointed structure within the epidermis directing downwards is consistent with the pilosebaceous unit. It is fully developed in adults, and accordingly, we did not find the structure in OCT images of juvenile skin.

The ET measured in this study was in accordance with that measured by another group [17] who included 12 coloured persons (skin types 4–6). We included 4 persons with skin types 4–5, and their ET did not differ from the persons with skin types 1–3, who constituted the majority of our sample. This is also in agreement with another study indicating that ET does not correlate with skin type [21]. Even though we only studied a small group of children, a statistically significant negative correlation between age (of all volunteers) and ET in all anatomical locations except the palm was demonstrated. The independence of palm ET may be explained by the keratinocyte activity in the palm. The SC is probably more affected by the physical activity of the hands than by age itself. We did not find gender differences in ET. One study found that the number of cell layers in the SC was independent of gender [39], another that males have a thicker cellular epidermis than females [21]. We did not find a significantly lower ET on the forehead of older women as in the study of Gambichler et al. [17]; however, our study population was smaller, and included fewer coloured volunteers and women. The small material can however be considered compensated by the morphological aspects of our study. No previous study has attempted to describe the qualitative regional variance of normal skin OCT morphology.

The PS-OCT images showed a well-demarcated difference between epidermis, papillary dermis and dermis. The actual dermo-epidermal junction could not be easily identified in PS-OCT images as it can in regular OCT images. This may be explained by the architecture of collagen in the papillary dermis: due to the loose structure the collagen tissue in the papillary dermis is less birefringent than in the reticular dermis. PS-OCT has shown potential in differentiating basal cell carcinoma from normal skin in pilot studies [25, 37]; however, the difficulties met in recognizing known anatomical structures in PS-OCT images must be solved before PS-OCT can be used in delineating and staging of skin tumours. Our study represents the first consecutive material of PS-OCT imaging of normal skin and also a necessary early step in the establishment of a new diagnostic imaging technique. A more detailed mapping of normal skin in OCT and PS-OCT images will allow for a more accurate diagnosis of pathologies and the possibility to monitor skin disease over time. We demonstrate age differences in the distinctive layering of sebaceous areas and heterogeneity of the pilosebaceous unit in OCT images that must be taken into account when interpreting OCT images. Furthermore we find that OCT has the ability to measure ET in an easily applicable and reliable way, and our results from OCT measurements are in accordance with ET measurements after cryopreservation. In comparison to in vivo confocal microscopy, OCT has an up to 7 times deeper penetration depth, a much wider field of view and provides cross-sectional imaging comparable to histopathology sections, but has a lower resolution. Compared to ultrasonography, OCT has an up to 50 times higher resolution at the cost of a lower penetration depth.

This study describes the baseline OCT and PS-OCT morphology of normal skin and indicates that OCT can be used for both the qualitative and quantitative assessment of skin structures.

References

1 Gambichler T, Moussa G, Sand M, et al: Applications of optical coherence tomography in dermatology. J Dermatol Sci 2005;40:85–94.
2 Marghoob AA, Swindle LD, Moricz CZ, et al: Instruments and new technologies for the in vivo diagnosis of melanoma. J Am Acad Dermatol 2003;49:777–797.
3 Jemec GB, Gniadecka M, Ulrich J: Ultrasound in dermatology. I. High frequency ultrasound. Eur J Dermatol 2000;10:492–497.
4 El Gammal S, El Gammal C, Altmeyer PJ, et al: High-resolution sonography of the skin; in Serup J, Jemec GBE, Grove GL (eds): Handbook of Non-Invasive Methods and the Skin, ed 2. Boca Raton, CRC Press, Taylor & Francis, 2006.
5 Kittler H, Pehamberger H, Wolff K, et al: Diagnostic accuracy of dermoscopy. Lancet Oncol 2002;3:159–165.
6 Zalaudek I: Dermoscopy subpatterns of non-pigmented skin tumors. Arch Dermatol 2005;141:532.
7 Gerger A, Koller S, Weger W, et al: Sensitivity and specificity of confocal laser-scanning microscopy for in vivo diagnosis of malignant skin tumors. Cancer 2006;107:193–200.
8 Liffers A, Vogt M, Ermert H: In vivo biomicroscopy of the skin with high-resolution magnetic resonance imaging and high frequency ultrasound. Biomed Tech (Berl) 2003;48:130–134.

9 Theumann NH, Goettmann S, Le VD, et al: Recurrent glomus tumors of fingertips: MR imaging evaluation. Radiology 2002;223: 143–151.
10 Lin SJ, Jee SH, Kuo CJ, et al: Discrimination of basal cell carcinoma from normal dermal stroma by quantitative multiphoton imaging. Opt Lett 2006;31:2756–2758.
11 Woodward RM, Cole BE, Wallace VP, et al: Terahertz pulse imaging in reflection geometry of human skin cancer and skin tissue. Phys Med Biol 2002;47:3853–3863.
12 Olmedo JM, Warschaw KE, Schmitt JM, et al: Optical coherence tomography for the characterization of basal cell carcinoma in vivo: a pilot study. J Am Acad Dermatol 2006;55:408–412.
13 Pierce MC, Strasswimmer J, Park BH, et al: Advances in optical coherence tomography imaging for dermatology. J Invest Dermatol 2004;123:458–463.
14 Welzel J: Optical coherence tomography in dermatology: a review. Skin Res Technol 2001;7:1–9.
15 Gluud C, Gluud LL: Evidence based diagnostics. BMJ 2005;330:724–726.
16 Welzel J, Reinhardt C, Lankenau E, et al: Changes in function and morphology of normal human skin: evaluation using optical coherence tomography. Br J Dermatol 2004;150:220–225.
17 Gambichler T, Matip R, Moussa G, et al: In vivo data of epidermal thickness evaluated by optical coherence tomography: effects of age, gender, skin type, and anatomic site. J Dermatol Sci 2006;44:145–152.
18 Fruhstorfer H, Abel U, Garthe CD, et al: Thickness of the stratum corneum of the volar fingertips. Clin Anat 2000;13:429–433.
19 Neerken S, Lucassen GW, Bisschop MA, et al: Characterization of age-related effects in human skin: a comparative study that applies confocal laser scanning microscopy and optical coherence tomography. J Biomed Opt 2004;9:274–281.
20 Gambichler T, Moussa G, Regeniter P, et al: Validation of optical coherence tomography in vivo using cryostat histology. Phys Med Biol 2007;52:N75–N85.
21 Sandby-Moller J, Poulsen T, Wulf HC: Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. Acta Derm Venereol 2003;83:410–413.
22 Olmedo JM, Warschaw KE, Schmitt JM, et al: Correlation of thickness of basal cell carcinoma by optical coherence tomography in vivo and routine histologic findings: a pilot study. Dermatol Surg 2007;33:421–425.
23 Gambichler T, Boms S, Stucker M, et al: Acute skin alterations following ultraviolet radiation investigated by optical coherence tomography and histology. Arch Dermatol Res 2005;297:218–225.
24 Gambichler T, Orlikov A, Vasa R, et al: In vivo optical coherence tomography of basal cell carcinoma. J Dermatol Sci 2007;45:167–173.
25 Strasswimmer J, Pierce MC, Park BH, et al: Polarization-sensitive optical coherence tomography of invasive basal cell carcinoma. J Biomed Opt 2004;9:292–298.
26 Barton JK, Gossage KW, Xu W, et al: Investigating sun-damaged skin and actinic keratosis with optical coherence tomography: a pilot study. Technol Cancer Res Treat 2003; 2:525–535.
27 Gambichler T, Hyun J, Moussa G, et al: Optical coherence tomography of cutaneous lupus erythematosus correlates with histopathology. Lupus 2007;16:35–38.
28 Gambichler T, Moussa G, Sand M, et al: Correlation between clinical scoring of allergic patch test reactions and optical coherence tomography. J Biomed Opt 2005;10:064030.
29 Welzel J, Bruhns M, Wolff HH: Optical coherence tomography in contact dermatitis and psoriasis. Arch Dermatol Res 2003;295: 50–55.
30 Morsy H, Mogensen M, Thrane L, Jemec GBE: Imaging of intradermal tattoos by optical coherence tomography. Skin Res Technol 2007;13:444–448.
31 Welzel J, Lankenau E, Birngruber R, et al: Optical coherence tomography of the skin. Curr Probl Dermatol 1998;26:27–37.
32 Morsy H, Mogensen M, Thomsen J, et al: Imaging of cutaneous larva migrans by optical coherence tomography. Travel Med Infect Dis 2007;5:243–246.
33 Gambichler T, Boms S, Stucker M, et al: Comparison of histometric data obtained by optical coherence tomography and routine histology. J Biomed Opt 2005;10:44008.
34 Tycho A, Andersen P, Thrane L, et al: Optical coherence tomography in dermatology; in Serup J, Jemec GBE, Grove GL (eds): Handbook of Non-Invasive Methods and the Skin, ed 2. Boca Raton, CRC Press, Taylor & Francis, 2006.
35 Fujimoto JG: Optical coherence tomography for ultrahigh resolution in vivo imaging. Nat Biotechnol 2003;21:1361–1367.
36 Gladkova ND, Petrova GA, Nikulin NK, et al: In vivo optical coherence tomography imaging of human skin: norm and pathology. Skin Res Technol 2000;6:6–16.
37 Strasswimmer J, Pierce MC, Park B, et al: Characterization of basal cell carcinoma by multifunctional optical coherence tomography. J Invest Dermatol 2003;121:0156.
38 Takiwaki H: Measurement of skin color: practical application and theoretical considerations. J Med Invest 1998;44:121–126.
39 Ya-Xian Z, Suetake T, Tagami H: Number of cell layers of the stratum corneum in normal skin – relationship to the anatomical location on the body, age, sex and physical parameters. Arch Dermatol Res 1999;291:555–559.