Correlation between clinical scoring of allergic patch test reactions and optical coherence tomography

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Abstract. Noninvasive imaging techniques might be of particular diagnostic value for studying and monitoring cutaneous inflammatory conditions such as contact dermatitis. We evaluate acute allergic contact dermatitis (AACD) by means of optical coherence tomography (OCT) and correlate the clinical grading of patch test reactions with the findings obtained from OCT. Twenty positive patch test reactions ($+, n=6; ++, n=7; +++, n=7$) are investigated using a conventional OCT scanner. In comparison to the control sites, OCT of AACD showed pronounced skin folds, thickened and/or disrupted entrance signals, and a significant increase in epidermal thickness. Moreover, clearly demarcated signal-free cavities within the epidermis and considerable reduction of dermal reflectivity are demonstrated by OCT. Notably, the latter findings strongly correlate with the clinical patch test grading. OCT may be a useful tool for visualization of micromorphological features of AACD. However, before OCT can be employed as an objective parameter in grading severity of patch test reactions, larger studies are required that correlate clinical patch test readings and OCT findings with histopathology. [DOI: 10.1117/1.2141933]

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1 Introduction

Allergic contact dermatitis is a significant cause of cutaneous disease affecting many individuals both at home and at work. In Germany, about 7% of the general population suffer from contact allergies,[1] a prevalence similar to that seen in America.[2] Patch testing is the most useful diagnostic tool for the evaluation of patients with suspected allergic contact dermatitis. Dermatologists routinely evaluate patch tests by means of visual inspection and palpatory findings. Typical clinical endpoints include erythema, infiltration, edema, and vesicle formation, some or all of which are rated on a simple scale[3,4] (e.g., 0, +, ++, +++). Such approaches can be criticized as subjective, of poor reproducibility, lacking in sensitivity, and being highly variable between observers and/or institutions. As a result, instrumental methods of assessment have been strongly promoted and do indeed offer several advantages, not least their objectivity.[5,6]

Numerous studies have been performed to find more objective methods of evaluating patch test reaction. For example, the following bioengineering methods have been assessed: transepidermal water loss measurement, laser Doppler flowmetry for measurement of cutaneous blood flow, assessment of erythema via colorimetry, IR thermography, and 20-MHz ultrasound A-scans to measure skin thickness.[5–7] However, innovative skin imaging techniques such as confocal laser scanning microscopy and optical coherence tomography (OCT) may be of particular interest because of the high resolution achieved by these techniques, which enable noninvasive visualization of micromorphological structures.[8,9] In contrast to ultrasound, OCT uses IR light instead of sound waves. It employs low-coherence interferometry to produce cross-sectional, 2-D or 3-D images of optical scattering from internal tissue microstructures. OCT enables imaging of skin layers up to 1 mm. Hence it is particularly suited for presenting morphological features of the epidermis and upper dermis. Briefly, interference fringes are formed when the optical path length of light reflected from the sample matches that given by the reference arm within the coherence length of the light source. The axial depth (A-scan) is obtained by scanning the reference arm length, resulting in localized interference fringes with amplitudes related to sample reflectivity. The fringe intensities in adjacent A-scans are combined to form a 2-D image (B-scan). The source coherence length and the diameter of the beam focus on the sample determine the depth resolution and lateral image resolution, respectively. A lateral resolution of the order of about 10 μm is typical for conventional OCT scanners. Furthermore, spectroscopic, elastographic, Doppler, and polarization-sensitive functions provide distinct, complementary information to conventional structural OCT. Importantly, there are no contraindications or adverse effects[9–13] for OCT.

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OCT is increasingly used in clinical and experimental dermatology. Correlation of OCT images with histology recently confirmed observation of anatomic structures such as different skin layers and appendages, including hair follicles and eccrine ducts. Pathological changes have also been described, such as blistering, tumor tissue, and inflammatory conditions including psoriasis and UV-induced dermatitis.[13–19] This explorative pilot study was designed to evaluate for the first time acute allergic contact dermatitis (AACD) by means of OCT, and to correlate the clinical grading of patch test reactions with the findings obtained from OCT.

2 Methods

2.1 *Patients and Patch Testing*

From October to November 2003, 68 patients with a clinical history of contact dermatitis routinely underwent standard patch testing in our department. The standard patch test on the upper back included 29 common allergens. Two test sites, one treated with petrolatum, one with aqueous, served as vehicle controls. The test was performed according to the German Contact Dermatitis Research Group (DKG) guidelines following international recommendations.[3,4] Small Finn Chambers® were used as carriers for the test substances. The application time of the patch test was 48 h. The tests were read after 48 h and 72 h. The allergic skin reactions were clinically graded as follows: 0=no reaction (negative); ?=macular erythema (doubtful); +=erythema, infiltration, discrete papules (weak allergic reaction); ++=erythema, infiltration, papules, vesicles (moderate allergic reaction); +++=erythema, infiltration, confluent vesicles (strong allergic reaction). Based on the 72-h test reading, we selected 10 patients (4 males and 6 females; median age 36.2 years) with a definite allergic skin reaction (+, ++, +++) to at least one allergen. After given informed consent, these patients were admitted to the study for consecutive OCT measurements.

2.2 *OCT Measurements*

Following the patch test reading, OCT measurements were performed on the skin sites with allergic reactions and the untreated control site. A commercial OCT scanner (SkinDex 300, ISIS optronics GmbH, Mannheim, Germany) was used in this study; this apparatus has been designed specially for imaging skin using a set of eight near-IR light-emitting diodes (LEDs). The eight LEDs are used to collect data simultaneously from eight channels. Regarding spatial resolution and field of view, the system functions are as follows. A bandwidth $\Delta\lambda=70$ nm and a center wavelength of $\lambda_0=1300$ nm were utilized. Assuming an average refractive index of the sample medium $n_{med}=n_{obj}=1.43$, this results in a coherence length for depth resolution $A\text{-FWHM}_{int}=7.4$ μm. The numerical aperture of the focusing lens is numerical aperture (NA)=0.19. Thus, the diffraction-limited lateral resolution yields $A\text{-FWHM}_{foc}=4.5$ μm. The architecture of the system with eight parallel scanning channels enables fast scans. Within 2 s, 512 scans are acquired along the length of 1 mm in lateral direction and an axial range of 0.9 mm. Echo signals are digitized with 14 bits amplitude resolution. The 3-D measurement modus of the SkinDex 300 with a 5-μm interplane distance was utilized to generate 15 2-D images.

The averaged A-scans were investigated to evaluate the amplitudes and presentation of the first and second peaks. From the B-scans, we selected one image per control site as well as patch test reaction showing the best quality, i.e., no artefacts. For example, we excluded images displaying the appearance of vertical bands that were due to mismatches in brightness and gain between the different LED channels of the SkinDex 300. Epidermal thickness (ET) was determined on the computer screen using a representative B-scan and the integrated measure tool (ruler) of the SkinDex 300. For this purpose, we manually measured on five predefined places in the OCT image: from the skin surface reflection (entrance signal) to the first well-demarcated change of reflectance intensity with clear echo-poor zone.[17,18] Image analysis was performed on the selected B-scans by viewing the images of interest on the screen side by side (control site versus patch test site). In all scans, we used the same image modalities (two-sided threshold operation: 60 dB/10 dB). The selected B-scans were investigated for the parameters as follows: thickening and/or splitting of the entrance signal; signal-free cavities within the epidermis; decrease of reflectivity in the upper dermis. To quantify the aforementioned parameters, we used the simple grading as follows: 0=none; 1=slight; 2=moderate; 3=strong. All OCT evaluations were performed by the same investigator who was blinded to the results of the patch tests.

2.3 *Statistics*

Statistical analysis was performed using Analyse-it (Analyse-it Software Ltd., Leeds, United Kingdom) Statistical Add-on for Excel (Microsoft, Redmond, Washington). Since data were nonnormally distributed, as confirmed by the Shapiro-Wilk test, correlations between the clinical patch test scoring and the OCT findings were analyzed employing the Spearman rank procedure. Differences of ET between patch test sites and control sites were assessed using the Wilcoxon signed ranks test. Coefficient of correlation (r), two-sided P-values for independent data sets, and confidence intervals (CI) were calculated. Differences were considered to be significant when $P<0.05$.

3 Results

In the 10 patients selected for further OCT assessments, we observed 20 allergic test reactions, for example, to p-phenylendiamine ($n=3$), nickel sulfate ($n=2$), colophony ($n=2$), and bufexamac ($n=2$). One patient had four positive patch test reaction, one patient had three, five patients had two, and the remaining three had one test reaction. All allergic reactions observed were caused by allergens that were prepared in petrolatum as a vehicle. In OCT images of the petrolatum-treated control sites, the skin surface showed a bright homogenous entrance signal without interruptions (first peak of A-scan). The superficial flat layer below the entrance signal corresponded to the epidermis. The border with dermis was usually sharply demarcated showing a highly reflective band of horizontally orientated collagen bundles (second peak of A-scan). Frequently, the dermis was more signal-intense than the epidermis. Some signal-free longish cavities, corresponding to blood vessels, were also observed in the dermis (Table 1). By contrast, OCT of AACD more frequently

Table 1 Data of OCT measurements in correlation to clinical patch test grading.

Patch Tests Clinical Grading	Mean Difference of ET (P-C)	OCT Grading#	Thickening/Splitting of the Entrance Signal*	Signal-Free Cavities in the Epidermis*	Decrease of Reflectivity in the Dermis*
		0	2	5	3
		1	2	1	2
+	11.5 μm	2	2	—	1
(n=6)		3	—	—	—
		0	2	—	—
		1	3	4	1
++	18.7 μm	2	2	2	4
(n=7)		3	—	1	2
		0	2	—	—
		1	4	—	—
+++	18.1 μm	2	1	1	2
(n=7)		3	—	6	5

ET, epidermal thickness; P-C, difference of ET between patch test site and control; #, 0=none; 1=slight; 2=moderate; 3=strong; * number of patch test reactions.

showed pronounced folding at the skin surface and a thickened and/or disrupted entrance signal mostly accompanied by splitting of the first peak of the A-scan. However, there was no correlation between the clinical scoring of AACD and the degree of thickening and/or splitting of the entrance signal observed in the B-scan (r=−0.16; 95% CI: −0.56 to 0.31; P=0.51). Evaluation of the A-scans of AACD revealed in almost all cases the loss of the second peak. Hence ET could be determined only in the B-scan using the SkinDex 300 measure tool. In allergen-treated skin (median ET: 77 μm) we observed a significantly ($P<0.001$; difference between medians: 13.6 μm; 95.2% CI: −18 to 10.5) thicker epidermis as compared to control sites (median ET: 65 μm). The increase of ET in allergen-treated skin did not correlate with the clinical scoring, however (r=0.29; 95% CI: −0.22 to 0.68; P =0.26). The most striking epidermal alteration found in AACD was the visualization of extensive, clearly demarcated signal-free cavities. The occurrence and size of signal-free cavities strongly correlated with clinical scoring (r=0.81; 95% CI: 0.57 to 0.93; $P<0.001$). While weakly positive allergic reactions usually showed no epidermal cavities in the OCT image, moderate and strong allergic reactions were accompanied by clearly demarcated cavities within the epidermis, partly even leading to an elevation of the skin surface [Figs. 1(c) and 1(d)]. In contrast to the control sites, allergic skin reactions displayed a remarkable decrease of reflectivity in the dermis. In extreme cases, there was an almost complete loss of signal intensity even in the upper dermis. The decrease of reflectivity strongly correlated with the clinical patch test scoring (r=0.74; 95% CI: 0.44 to 0.89; $P<0.001$).

4 Discussion

Investigation of AACD is of significant interest in dermatology and occupational medicine. However, most current methods to evaluate AACD are limited by the use of indirect techniques that may be inaccurate or insensitive. Noninvasive imaging techniques for the investigation of AACD enable the skin to be imaged as frequently as required. Standard noninvasive methods such as magnetic resonance imaging and ultrasound, however, provide fairly undifferentiated information.[1–6] By contrast, OCT is capable of yielding more micromorphological details than the other methods mentioned. Therefore, it may be a promising tool for the evaluation of inflammatory skin conditions. Pagnoni et al.[14] previously investigated the effect of dimethyl sulfoxide on human skin using a prototype of the SkinDex 300. They observed an increase in surface folding, a dark vacuolization within the epidermis, and hyporeflectivity and decrease of signal attenuation in the dermis. The latter was also observed following the application of histamine or nictotinic acid.[16] Recently, sodium lauryl sulfate-induced contact dermatitis has been studied by Welzel et al.[9] using OCT *in vivo*. Following skin irritation with sodium lauryl sulfate, they observed larger and more irregular superficial skin folds, a more pronounced entrance signal, increase of ET, and dilated blood vessels in the dermis. Comparison of the aforementioned findings with our results is however difficult, since Welzel et al.[9] studied an experimental model of acute irritant contact dermatitis (AICD) and did not report on the clinical grading of skin reactions. Recently, it has been shown in confocal microscopy studies *in vivo* that

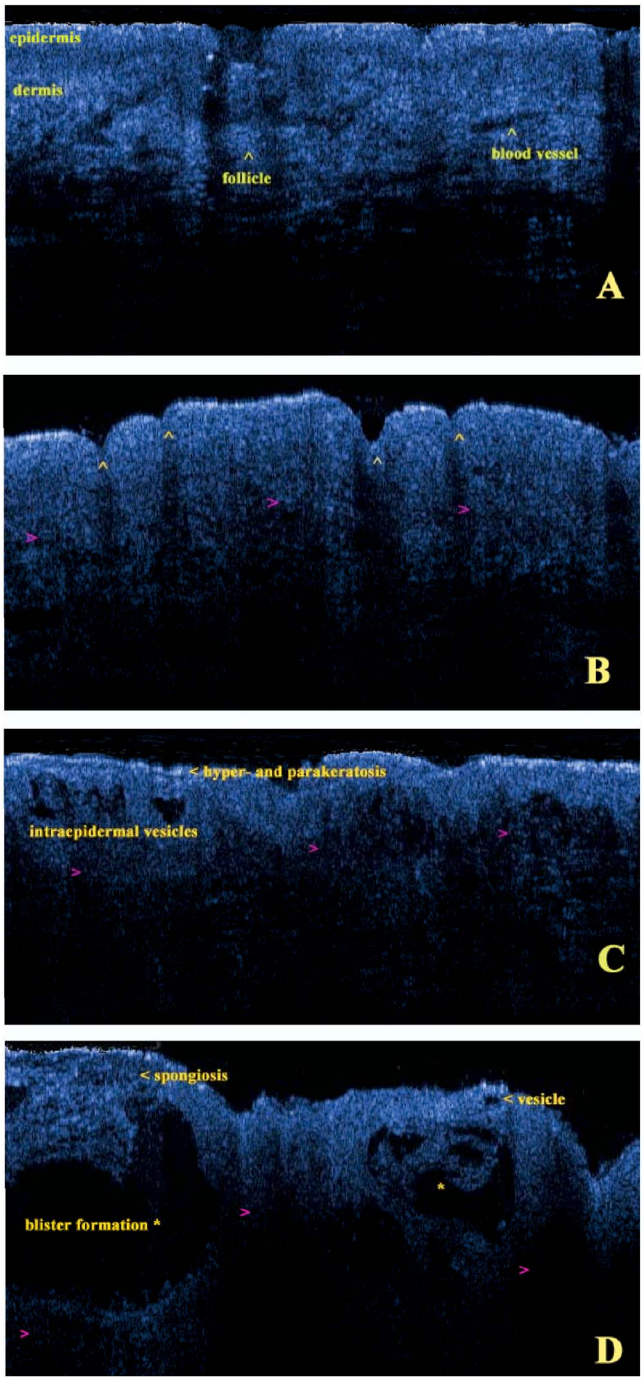

Fig. 1 OCT images of a patient who had several positive patch test reactions. OCT shows a typical presentation of normal skin with a relatively clear demarcation of the epidermis and dermis. The latter displays strongly reflective, partly horizontally arranged structures corresponding to collagen bundles (control, A). The B-scan of the weak patch test reaction shows more pronounced folding at the skin surface (∧). In comparison to the control site, a reduction of reflectivity in the dermis is evident (>). Furthermore the border between the epidermis and dermis appears more diffuse (+, lanolin alcohol, B). The image of the moderate test reaction demonstrates signal-free epidermal cavities (vesicles), thickening and splitting of the entrance signal (focal hyper- and parakeratosis), and considerable reduction of dermal reflectivity (++, ammoniated mercury, C). The OCT scan of the strong test reaction clearly demonstrates small to large signal-free epidermal/dermal cavities (spongiosis, vesicles, blisters). An almost complete loss of dermal reflectivity possibly due to edema is evident as well (+++, bufexamac, D).

superficial epidermal changes, including striking parakeratosis, stratum corneum disruption, and superficial necrosis, are highly indicative of irritant reactions. Though an increase of ET may be observed in AACD, the degree is usually rather mild as compared to AICD. On the other hand, vesicle formation and edema in the upper dermis appear to be more severe in AACD than in AICD. Hence the differentiation between AACD and AICD might be possible by means of confocal microscopy *in vivo*.[8,19] Nevertheless, even the value of histological examination to differentiate between the distinctive forms of contact dermatitis is limited.[20,21]

In this study, we found that AACD tended to generate larger folds at the skin surface probably due to edema in the epidermis. Further, we observed thickening and/or splitting of the entrance signal possibly indicating slight to moderate hyper- and parakeratosis caused[9] by AACD [Fig. 1(c)]. This finding, however, did not correlate with the clinical scoring. The stratum corneum of body skin is normally not visible on conventional OCT. However, in particular pathological circumstances such as hyper- and parakeratosis occurring in psoriasis or sunburn, the stratum corneum may be resolved by means of commercially OCT scanners.[9,17] Even though statistically significant, the increase of ET (difference between medians: 13.6 μm) observed in AACD was moderate from a clinical point of view and did not correlate with clinical severity. We presume that the increase of ET following AACD may be due to intercellular edema as well as hyperproliferative processes induced by the release of inflammatory cytokines.[4,9]

The most striking finding of the present study represents the visualization of well-demarcated signal-free cavities within the epidermis.[22] These findings very likely correspond to coalescing spongiosis and vesicle formation which is a typical feature[4,20,21] of AACD. Moreover, we observed a strong correlation between the clinical scoring and decrease of reflectivity in the dermis. Possibly, the latter can be explained by a reduction of backscattering in the upper dermis due to interstitial edema, cellular infiltrates, and dilation of blood vessels. Hence within the patch test reactions, the dermis appeared much darker due to reduced scattering. The latter is mainly influenced by the size, shape, and composition of particles. The regular arrangement of collagen in the upper dermis leads to a strong backscattering.[18,19] Any alteration in the orientation of the collagen fibers or the structural composition of the dermis influences its optical properties. As in our data, Raju et al.[7] recently observed in a high-frequency ultrasound study on patch test reactions a significant increase in skin thickness and decrease in echogenicity of the upper dermis.

The major limitation of the presented study, however, is that there are no biopsies for histopathologic confirmation of our findings. For example, whether the regions with clear demarcation and reduced reflecting signals are due to vesiculation, or if the increase of ET is due to edema and hyperproliferation is more or less based on speculations only indirectly supported by previous OCT studies including histologic investigations.[9,13,18,22–24] Since the clinical patch test readings have major limitations, the study lacks of a "gold standard" by which to compare the accuracy and validity of OCT readings. Therefore, it remains uncertain whether the concordance or discordance between OCT and clinical evaluation adds value to diagnosis of AACD. In conclusion, we demonstrated

definite OCT findings in patients with AACD including pronounced skin folding, thickening, or disruption of the entrance signals, and increase in ET. Moreover clearly demarcated signal free cavities and reduction in dermal reflectivity were detected by OCT which significantly correlated with clinical patch test grading. Even though OCT appears to be a useful technique to evaluate AACD, clinical correlations in the future will be needed to determine the true value of OCT in this setting.[25]

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References

1. G. Heine, A. Schnuch, W. Uter, and M. Worm, "Frequency of contact allergy in German children and adolescents patch tested between 1995 and 2002: results from the Information Network of Departments of Dermatology and the German Contact Dermatitis Research Group," *Contact Dermatitis* **51**, 111–117 (2004).
2. J. G. Marks et al., "North American Contact Dermatitis Group patch test results for the detection of delayed-type hypersensitivity to topical allergens," *J. Am. Acad. Dermatol.* **38**, 911–918 (1998).
3. A. Schnuch, W. Aberer, M. Agathos, J. Brasch, P. J. Frosch, T. Fuchs, and G. Richter, "Leitlinien der Deutschen Dermatologischen Gesellschaft (DDG) zur Durchführung von Epikutantests mit Kontaktallergenen," *Hautarzt* **52**, 864–866 (2001).
4. R. L. Rietschel, J. F. Fowler, A. A. Fisher, Eds. *Fisher's Contact Dermatitis*, 5th ed., Lippincott Williams & Wilkins, Baltimore, MD (2001).
5. T. Agner and J. Serup, "Individual and instrumental variations in irritant patch-test reactions–clinical evaluation and quantification by bioengineering methods," *Clin. Exp. Dermatol.* **15**, 29–33 (1990).
6. V. Zuang, G. Archer, C. Rona, M. Vignini, M. Mosca, and E. Berardesca, "Predicting visual assessment of allergic patch test reactions by non-invasive measurements," *Skin Pharmacol. Appl. Skin Physiol.* **13**, 39–51 (2000).
7. B. I. Raju, K. J. Swindells, S. González, and M. A. Srinivasan, "Quantitative ultrasonic methods for characterization of skin lesions in vivo," *Ultrasound Med. Biol.* **29**, 825–838 (2003).
8. K. Swindells, N. Burnett, F. Rius-Diaz, E. Gonzalez, M. C. Mihm, and S. Gonzalez, "Reflectance confocal microscopy may differentiate acute and irritant contact dermatitis in vivo," *J. Am. Acad. Dermatol.* **50**, 220–228 (2004).
9. J. Welzel, M. Bruhns, and H. H. Wolff, "Optical coherence tomography in contact dermatitis and psoriasis," *Arch. Dermatol. Res.* **295**, 50–55 (2003).
10. J. G. Fujimoto, "Optical coherence tomography for ultrahigh resolution in vivo imaging," *Nat. Biotechnol.* **21**, 1361–1367 (2003).
11. W. Drexler, "Ultrahigh-resolution optical coherence tomography," *J. Biomed. Opt.* **9**, 47–74 (2004).
12. B. Hyle Park, M. C. Pierce, B. Cense, and J. F. de Boer, "Real-time multi-functional optical coherence tomography," *Opt. Express* **11**, 782–793 (2003).
13. M. C. Pierce, J. Strasswimmer, B. Hyle Park, B. Cense, and J. F. de Boer, "Advances in optical coherence tomography imaging for dermatology," *J. Invest. Dermatol.* **123**, 458–463 (2004).
14. A. Pagnoni, A. Knuettel, P. Welker, M. Rist, T. Stoudemayer, and L. Kolbe, "Optical coherence tomography in dermatology," *Skin Res. Technol.* **5**, 83–87 (1999).
15. J. Weissman, T. Hancewicz, and P. Kaplan, "Optical coherence tomography of skin for measurement of epidermal thickness by shapelet-based image analysis," *Opt. Express* **12**, 5760–5769 (2004).
16. J. Welzel, C. Reinhardt, E. Lankenau, C. Winter, and H. H. Wolff, "Changes in function and morphology of normal human skin: evaluation using optical coherence tomography," *Br. J. Dermatol.* **150**, 220–225 (2004).
17. T. Gambichler, S. Borns, M. Stücker, G. Moussa, A. Kreuter, M. Sand, D. Sand, P. Altmeyer, and K. Hoffmann, "Acute skin alterations following ultraviolet radiation investigated by optical coherence tomography and histology," *Arch. Dermatol. Res.* **297**, 218–225 (2005).
18. T. Gambichler, S. Boms, M. Stücker, A. Kreuter, M. Sand, G. Moussa, P. Altmeyer, and K. Hoffmann, "Comparison of histometric data obtained by optical coherence tomography and routine histology," *J. Biomed. Opt.* **10**(4), 644008 (2005).
19. S. Neerken, G. W. Lucassen, M. A. Bisschop, E. Lenderink, and T. A. Nuijs, "Characterization of age-related effects in human skin: a comparative study that applies confocal laser scanning microscopy and optical coherence tomography," *J. Biomed. Opt.* **9**, 274–281 (2004).
20. L. Vestergaard, O. J. Clemmensen, F. B. Sorensen, and K. E. Andersen, "Histological distinction between early allergic and irritant patch test reactions: follicular spongiosis may be characteristic of early allergic contact dermatitis," *Contact Dermatitis* **41**, 207–210 (1999).
21. D. L. Jovanović, A. Petrović, M. Paravina, M. Stanojević, and I. Binić, "Chronic contact allergic and irritant dermatitis of palms and soles: routine histopathology not suitable for differentiation," *Acta Dermat.* **12**, 127–130 (2003).
22. J. Welzel, E. Lankenau, R. Birngruber, and R. Engelhardt, "Optical coherence tomography of the human skin," *J. Am. Acad. Dermatol.* **37**, 958–963 (1997).
23. J. Strasswimmer, M. C. Pierce, B. H. Park, V. Neel, and J. F. de Boer, "Polarization-sensitive optical coherence tomography of invasive basal cell carcinoma," *J. Biomed. Opt.* **9**, 292–298 (2004).
24. V. de Giorgi, M. Sante, D. Massi, L. Mavilia, P. Cappugi, and P. Carli, "Possible histopathologic correlates of dermoscopic features in pigmented melanocytic lesions identified by means of optical coherence tomography," *Exp. Dermatol.* **14**, 56–59 (2005).
25. T. Gambichler, G. Moussa, M. Sand, D. Sand, P. Altmeyer, and K. Hoffmann, "Applications of optical coherence tomography in dermatology," *J. Dermatol. Sci.* **40**, 85–94 (2005).