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**CLINICAL APPLICATIONS AND HISTOPATHOLOGIC CORRELATIONS
OF LINE-FIELD CONFOCAL OPTICAL COHERENCE TOMOGRAPHY IN
DERMATOLOGY**

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ABSTRACT (ENGLISH)

Background

Imaging tools gained increased popularity in the clinical practice. Besides confocal microscopy and optical-coherence-tomography, the novel device line-field confocal optical-coherence-tomography (LC-OCT) was developed for bedside diagnosis of skin lesions at cellular resolution and penetration depth up to the mid-dermis.

Objectives

To investigate novel applications, standardized descriptors and diagnostic sensitivity and specificity of LC-OCT for healthy skin, skin tumors and inflammatory skin diseases compared to the gold standard histology.

Methods

Six-hundred skin lesions were included in the study so far. Clinical, dermoscopic, imaging pictures were collected. When excision was indicated, the histological examination was performed. Diagnostic criteria were assessed and compared with histology.

Results

Main LC-OCT features of basal cell carcinoma (BCC) were: atypical keratinocytes, altered DEJ, tumor nests in the dermis, dark clefting, prominent vascularisation and white hyperreflective stroma for nodular BCCs, string of pearls pattern for superficial BCCs, shoal of fish pattern for infiltrative BCCs. Overall BCC subtype agreement LC-OCT vs histology was 90.4 % (95% CI: 79.0, 96.8). Main patterns of keratinocyte cancer (KC) were hyperkeratosis/parakeratosis, disruption of stratum corneum, broadened epidermis, keratinocyte atypia, dilated vessels and collagen alterations. LC-OCT grading of AK based on the PRO classification agreed with the histological in 75% lesions. A deep learning software reached over 71% agreement with the operator-dependent score. In melanocytic lesions, no difference in performance between RCM and LC-OCT was observed. The most significant criteria for diagnosing a melanoma with LC-OCT were an irregular honeycombed pattern, pagetoid spread and the absence of dermal nests. Good image quality correlated with better diagnostic performance.

Conclusions

LC-OCT can enrich the actual knowledge on the in-vivo analysis of healthy skin and skin diseases. Further studies including melanoma and inflammatory skin diseases are needed to gain more experience in this yet unexplored field of dermatology.

Premessa

Gli strumenti di indagine non invasiva della cute hanno acquisito una crescente popolarità nella pratica clinica. Oltre alla microscopia confocale e alla tomografia a coerenza ottica, è stato sviluppato un nuovo dispositivo, la tomografia a coerenza ottica confocale a scansione lineare (LC-OCT), per la diagnosi di lesioni cutanee con risoluzione cellulare e profondità di penetrazione fino al derma.

Obiettivi

Analizzare le applicazioni cliniche, la sensibilità e specificità diagnostica della LC-OCT per la cute sana, i tumori cutanei e le malattie infiammatorie della pelle rispetto al gold standard istologico. Identificare le correlazioni morfologiche tra LC-OCT e istologia ed elaborare una nomenclatura standardizzata per i pattern individuati.

Metodi

Nello studio sono state incluse finora seicento lesioni cutanee. Sono state raccolte immagini cliniche, dermoscopiche e di imaging. Quando era indicata l'escissione, è stato eseguito l'esame istologico. I criteri diagnostici sono stati valutati e confrontati con l'istologia.

Risultati

Le principali caratteristiche LC-OCT del carcinoma basocellulare (BCC) sono state: cheratinociti atipici, alterata giunzione dermo-epidermica, presenza di nidi tumorali nel derma, clefting peritumorale, vascolarizzazione prominente e reazione stromale iper-riflettente per i BCC nodulari, struttura a filo di perle per i BCC superficiali, struttura a banco di pesci per i BCC infiltranti. La concordanza riguardo il sottotipo di BCC tra LC-OCT e istologia è stata del 90,4% (95% CI: 79,0, 96,8). Le principali caratteristiche dei tumori cheratinocitari erano ipercheratosi/paracheratosi, interruzione dello strato corneo, epidermide allargata, atipia dei cheratinociti, vasi dilatati e alterazioni del collagene. La classificazione LC-OCT della cheratosi attinica basata sulla classificazione PRO concordava con quella istologica nel 75% delle lesioni. Un software di deep learning ha raggiunto una concordanza di oltre il 71% con quella definita dal clinico. Nelle lesioni melanocitarie non è stata osservata alcuna differenza nelle prestazioni diagnostiche tra RCM e LC-OCT. I criteri più significativi per la diagnosi di melanoma con LC-OCT sono stati un pattern irregolare a nido d'ape, la crescita pagetoide e l'assenza di nidi dermici. Una buona qualità dell'immagine è stata correlata a una migliore performance diagnostica.

Conclusioni

La LC-OCT può arricchire le attuali conoscenze sull'analisi non invasiva della cute sana e delle malattie cutanee. Sono necessari ulteriori studi che includano il melanoma e le malattie infiammatorie della pelle per acquisire maggiore esperienza in questo campo ancora inesplorato della dermatologia.

BACKGROUND

NON-INVASIVE DIAGNOSTICS IN DERMATOLOGY

WIDESPREAD IMAGING METHODS

The development of non-invasive diagnostic techniques in dermatology significantly changed the dermatological clinical routine in the last two decades. Dermoscopy, a cheap and light portable tool every dermatologist can't now do without, was the first device introduced in the nineties. Until then, malignant melanoma (MM) could only be diagnosed at advanced stage, while now even in-situ (early, intraepithelial) tumors can often be recognized and excised before they reach a deadly stage. Using dermoscopy, clinicians drastically increased their diagnostic sensitivity and specificity compared to naked eye examination, not only for MM but also for non-melanoma skin cancer (NMSC).[1,2]

In addition to dermoscopy, more sophisticated technologies such as optical coherence tomography (OCT) and reflectance confocal microscopy (RCM) made their way from research tools to daily routine instruments for diagnosis and follow-up of skin cancer and even inflammatory skin diseases, hair diseases or parasitic infestations.[3] Both of them are painless, quick and allow a diagnostic assessment at bedside, with good and intuitive correlations to histopathology.[4]

Over and above the improvement of pure visual diagnostics, the immediate purposes of such modern diagnostic tools are: pre-operative tumor mapping with margin control, assessment of recurrences and success of local, non-surgical treatments. Not only skin cancer, but also inflammatory and blistering diseases as well as parasitic infestations can be better investigated by means of non-invasive diagnostic techniques.[5,6] Furthermore, clinical research can significantly benefit of such tools in order to investigate cellular and architectural changes in the skin due to physiological processes such as photoaging or hydration.[7-11]

DERMOSCOPY

The introduction of dermoscopy, a completely noninvasive method, into clinical practice has made it possible to discover a new morphological dimension of skin pigmented lesions, partly overcoming the diagnostic difficulties posed by observation with the naked eye alone. Surface microscopy is now fundamental for the dermatology specialist, who uses portable instruments (dermoscopes or dermoscopes) that exploit the phenomenon of epiluminescence. This technique exploits the emission of polarized light together with a thin film of liquid (water, vaseline oil or alcohol) that prevents the normal scattering of light (light scattering) to the stratum corneum alone, reduces its reflection index and allows clear visualization of the underlying structures down to the superficial dermis. Also among the instruments available to the physician are videodermoscopes, which are associated with a camera connected to a computerized device; this system allows evaluation of the pigmented lesion on a screen, as well as the recording of images that can be archived and consulted later during patient follow-up.

Dermoscopy has over time become essential for the diagnosis of MM: this skin cancer is relatively infrequent but melanocytic nevi, which are the lesions with which the differential diagnosis should be made, are extremely common. Distinguishing benign and malignant melanocytic lesions by clinical examination alone can be quite complex; dermoscopy provides significant help in this regard. There is, in fact, a correlation between dermoscopic features and histologic structures, despite the fact that the two examinations take place on different spatial planes: dermoscopic observation on a horizontal plane, histologic observation on a sagittal plane.

The two main features considered by dermoscopic parameters are morphology and color. It is the melanin pigment that is primarily responsible for the visibility of a lesion; the color of melanin depends on the depth of its localization within the skin. Main dermoscopic features of melanocytic lesions are: pigmentary reticulum, black dots, brown globules, radial striae, pseudopodia, homogeneous diffuse pigmentation, areas of regression, whitish blue veil, and vascular pattern. (Figure 1) It should be mentioned that non-melanocytic lesions also possess specific dermatoscopic

features. BCC is typically characterized by arborizing vessels, blue gray ovoid nests, ulceration (nodular BCCs), superficial fine telangiectasias, spoke wheel structures, leaf like structures, blue gray or brown dots, shiny white blotches and strands (superficial BCCs), structureless hypopigmentation or porcelain white areas (sclerosing BCC). On the contrary, AK typically presents in its early stages with a red pseudonetwork (strawberry pattern), progressing with an increasing neovascularization (development of clustered dotted/glomerular vessels) to Bowen disease (BD). The follicles gradually miniaturize and disappear, whereas hairpin and linear-irregular vessels appear. Along with these vascular changes, a central mass of keratin forms and ulceration may occur (SCC).

The use of dermoscopy is associated with a significant increase in sensitivity in the diagnosis of melanoma and a significant reduction in the number of benign lesions improperly excised. Scientific studies have demonstrated an increase in diagnostic accuracy for pigmented lesions: a systematic review calculated a 90% sensitivity of dermoscopic diagnosis, compared with a 74% sensitivity of naked-eye examination. Cochrane reviews[12] confirmed that when performed by trained physicians, dermoscopy detects melanoma better than naked-eye examination. This is reflected in a decrease in the percentage of patients undergoing biopsies of suspicious lesions, thus improving specificity, as well as facilitating in the detection of thinner tumors.[12,13]



Figure 1. Clinical (A) and dermoscopic image 100x (B, C) and 20x (A) of an *in-situ* melanoma arising on a compound nevus, with atypical pigment network, atypical dots, blue-whitish veil and regression features. Acquired with the digital dermoscope FotoFinder (FotoFinder Systems GmbH, Bad Bimbach, Germany)

OPTICAL COHERENCE TOMOGRAPHY

OCT has a good penetration depth to the deep dermis, but at the expense of lower resolution, and is therefore mainly used for NMSC.[14] In contrast, RCM is particularly suitable for the differential diagnosis of melanocytic lesions thanks to its resolution at the cellular level.[15] It can also be used ex-vivo (on excised tissue directly postoperatively) to provide a virtual, faster type of histology.[16,17]

OCT has been used in ophthalmology for several years, and was later borrowed by dermatologists and adapted to their own needs in order to better fit skin diagnostics. OCT is based on coherent light interferometry. Live in-vivo imaging of the skin down to subcutaneous tissue (1-2 mm penetration depth) is possible, but with a lower resolution ($\approx 10\text{-}20\ \mu\text{m}$) compared to RCM. The images are both vertical and horizontal, in grayscale. The dynamic function displays blood vessels in red color.

The current commercially available model worldwide is the OCT device VivoSight[®] (Michelson Diagnostics Ltd., Maidstone, Kent, England). Other devices have been used in the research field but are not commercially widespread.

The OCT images can be interpreted with basic histological knowledge, as they appear in vertical sections, i.e., the same perspective of histological slides. In the vertical section, the broad *stratum corneum* (SC) can be seen, interrupted by sweat gland excretory ducts. The underlying *stratum granulosum/ spinosum* is separated from the superficial dermis by a narrow dark line, the dermo-epidermal junction (DEJ). The dermis is richer in signals in the *stratum papillare*, but becomes poorer in signals in the *stratum reticulare*, where vessels can be delineated as dark, round or elongated structures.

The main indication of OCT has been NMSC. Of course, numerous skin conditions can potentially be examined (eczema, rosacea, scarring, skin hydration), although such indications are mainly confined to academic purposes. In particular, field carcinogenesis (FC), referring to the continuum of NMSC from early subclinical to advanced stages, can be characterized with high sensitivity and specificity. Actinic keratosis (AK), the earliest stage of keratinocyte skin cancer, shows inhomogeneous thickening of the *stratum corneum*, which can be measured on the display, together with acanthotic thickening of the rest of the epidermis. Increasing atypia is seen in squamous cell carcinoma (SCC) not only as hyperkeratosis but also with loss of structure of the normal epidermal and junctional structure.[18,19]

Basal cell carcinoma (BCC) is, on the contrary, characterized by an atrophic epidermis and the typical sharply delimited, signal-poor, ovoid or round tumor nodules. Sometimes there are central cystic portions in nodular forms. Superficial BCCs show interconnected, linear and ovoid structures described as string-of-pearls pattern, while fibrosing BCCs are more difficult to diagnose: the diagnostic clue is often a kind of "shoal of fish" pattern of inhomogeneous, elongated, hyporeflective structures.[20]

OCT is an operator-dependent technique and the experience of the assessing physician influences the image interpretation. To partially overcome this disadvantage and to be able to examine skin

morphology in a standardized way, especially in clinical studies, a special software was developed. VivoTools® analyses the OCT images of the skin and determines a number of objective parameters that provide significant information about the skin morphology. In particular, it is possible to determine: blood vessel density as a function of depth, roughness of the skin, epidermis thickness and optical skin attenuation coefficient (AC). Furthermore, hair folds, hair follicles and pores can be displayed in 2D and 3D. The software is still in the validation phase, but has shown promising results in our experience; it could be used specifically in the comparative studies to evaluate the changes in the above skin parameters before, during and after treatments with cosmetics and local therapeutics.[21-23] (Figure 2)

OCT has been used mainly for the diagnosis of NMSC. Trained physicians reached a sensitivity of 86–95% and a specificity of 81–98% in diagnosing BCC using OCT, with a significant improvement compared to dermoscopy.[20] OCT is also successful in diagnosing the spectrum of field cancerization, with reported sensitivity and specificity of 93-98% for SCC diagnosis and 81-92% for AK diagnosis.[18,24,25] The lower resolution of OCT compared to RCM makes the diagnosis of melanocytic lesions very challenging. Dynamic OCT has shown some potential in this field, although confined to an academic context [26].

In an attempt to combine the advantages of RCM (cellular resolution) and OCT (penetration depth), a new diagnostic method has been developed in recent years: line-field confocal optical coherence tomography (LC-OCT). The LC-OCT device (DAMAE Medical, Paris) combines the principle of OCT interferometry with the spatial filtering of RCM, providing three in vivo imaging modalities: histological-like vertical, RCM-like horizontal and a new unique 3-D reconstruction. An axial resolution of 1.1 μm , a lateral resolution of 1.3 μm and a penetration depth of $\sim 500 \mu\text{m}$ make LC-OCT ideal for the diagnosis and monitoring of skin tumors. Despite being a relatively new diagnostic method, some authors have already evaluated its usefulness in the diagnosis of different skin tumors including AK and SCC, and in the diagnosis and monitoring of BCC and pigmented lesions.

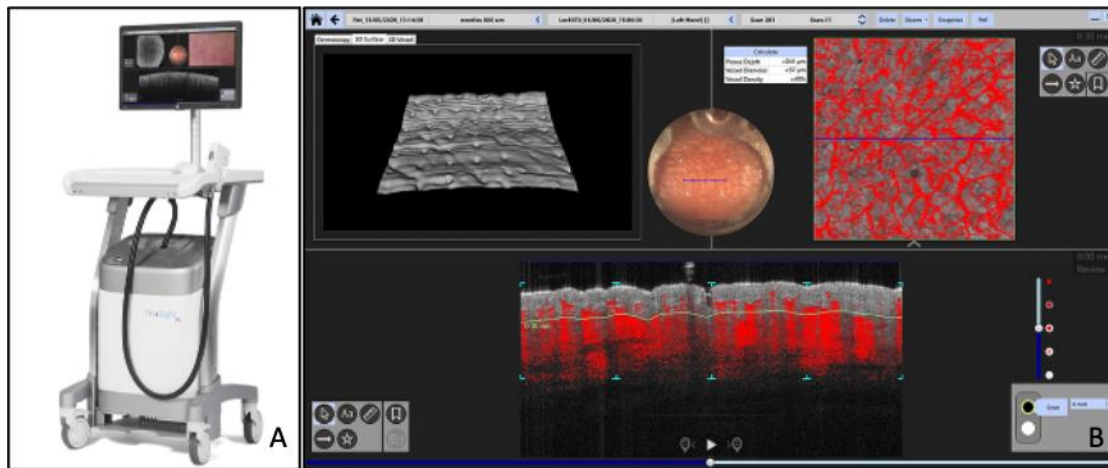


Figure 2. Commercially available OCT device Vivosight (Michelson Diagnostic, Maidstone, Kent, UK) (A), with user screen showing from left above to right below: 3D reconstruction, camera image, horizontal section with dynamic vessel function and vertical section with dynamic vessel function of healthy skin (<https://us.vivosight.com/vivosight-dx/>)

REFLECTANCE CONFOCAL MICROSCOPY

The systematic use of RCM in dermatology dates back to the 1990s at Memorial Sloan Kettering Hospital in New York. [27] It originated as an ex vivo technique for skin samples and was first discovered for in vivo applications. The ex vivo field of surgically harvested tissue samples has been further developed in recent years with great success.[28,29]

RCM is an optical device based on an infrared laser light source (wavelength 830nm). An integrated camera allows examination of both macroscopic images of the skin surface and dermoscopy images of lesions. This implies live navigation through the lesion. Two of his models are available, manufactured by Mavig GmbH (Munich, Germany).

Vivascope© 1500 and a more compact handheld version (Vivascope© 3000). RCM images are horizontal (parallel to the skin surface) and black and white. The methodology does not require contrast agents: contrast is created by varying intensities of reflection of the skin's natural chromophores such as keratin and melanin. Due to their relatively high refractive index compared to air and water, the pigmented cells appear very bright, while the aqueous cytoplasm appears dark. Capture the epidermis, dermo-epidermal junction (DEJ), and epidermis using a standard three-layer image. The device is not suitable for thicker stratum corneum or deeper structures, as the image

blurs from a depth of 200-250 μm , but it offers a resolution of 1.25 μm that allows visualization of individual cells. RCM is particularly suitable for diagnosing melanocytic tumors as it may show cellular atypia. It is also commonly applied to epithelial skin tumors such as AK, SCC and BCC.

Comparison with histology (longitudinal section) takes some getting used to, as it is a horizontal image. Corneocytes from the SC appear as adjacent large polygonal cells and are uniformly bright. Below are the stratum granulosum and stratum spinosum, which correspond to a carpet of similarly sized polygonal keratinocytes with light borders and black cytoplasm. This creates polygonal structures called "honeycombs" that correspond to honeycombs found in nature. In DEJ, light-colored basal keratinocytes and melanocytes form a sort of ring around the darker skin papillae and cut horizontally (a 'ring pattern'). Capillaries are visible in the center of the papilla. In vivo visualization of blood flow is possible. The superficial dermis contains mainly blood vessels and collagen fibers. At depth, RCM continuously loses resolution. [30-34] (Figure 3)

RCM can successfully be employed in the daily clinical practice for the diagnosis of melanocytic and non-melanocytic skin lesions as well as for perioperative mapping and follow up of NMSC, using established diagnostic criteria associated to high diagnostic sensitivity and specificity. The reported sensitivity and specificity of RCM for diagnosing MM, BCC, and SCC was around 80–100%. [25-27] RCM is also suitable for non-invasive characterization of numerous skin diseases such as psoriasis, eczema, bullous skin disease, and parasitic infections, even though still confined to the academic field. [28,29]

On dermoscopically equivocal pigmented lesions, combined RCM and dermoscopy examination allowed a sensitivity of 97.8% and a specificity of 92.4% for diagnosing melanoma, compared to dermoscopy alone (94.6 and 26.74%, respectively). [35] Also in the setting of high risk patients, the sensitivity and specificity for the diagnosis of melanoma using RCM were 100% and 69%. [36]

The diagnostic sensitivity and specificity for BCC and dermoscopy by RCM reached a sensitivity of 100% and specificity of 78%[37] . in general, meta-analysis found RCM to be more sensitive but less specific for the detection of BCC in studies comprehending equivocal lesions (sensitivity 94%, 95%; specificity 85%, 95%) compared to those without (sensitivity 76%, 95%, specificity 95%, 95%).[38]

In a setting of mixed lesions, with the aim of determining the potential role of RCM in daily clinical practice and assessing how many unnecessary excisions could be avoided in order to distinguish among MM, BCC and benign lesions, combined RCM and dermoscopy reached a sensitivity of 100% and a specificity of 80.21%, compared to dermoscopy alone (100% and 51.77%).[39]

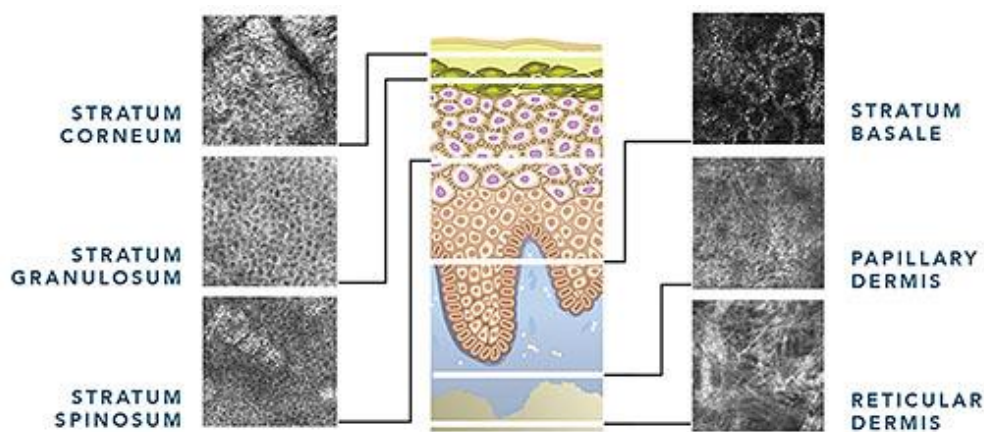


Figure 3. Correlation of RCM images to the corresponding skin layers, adapted from <https://caliberid.com/vivascope-system.html>

HIGH FREQUENCY ULTRASONOGRAPHY

Although above-described tools are the most commonly used in the dermatological praxis nowadays, there are still other methods worth to be mentioned. Ultrasonography is a quick, non-invasive diagnostic device broadly used in most medical fields. Ultrasound imaging is based on the principle of wave reflection on interfaces with acoustic impedance. A transducer (containing piezoelectric crystals) is applied to the skin, generates sound waves, and receives feedback waves converted into electrical signals, which are processed and displayed as an image. The color Doppler function allows the visualization of vascular morphology and blood flow in real time.

High frequency ultrasonography (HFUS) has higher resolution than conventional ultrasound, which is directly proportional to depth of penetration and inversely proportional to frequency. The most commonly used frequencies in dermatology are between 20 and 50 MHz. HFUS has found a niche in the characterization of fibrosing skin diseases such as scleroderma, and was even used in few studies for non-invasive skin cancer diagnosis, tumor depth measurement, preoperative mapping of skin neoplasm margins, loco-regional staging, tumor recurrence detection, follow-up of local treatments.

Skin cancers appear as hypoechoic areas on ultrasound imaging. SCC has been differentiated from BCC because of its more prominent vascularity; also, melanoma has a prominent vascular pattern and marked skin invasion. Such features are however insufficient to provide a specific differential diagnosis, so that the device only plays a role in dermato-oncology for the assessment of tumor size and depth.[40-48]

EMERGING IMAGING TECHNIQUES

LINE-FIELD CONFOCAL OPTICAL COHERENCE TOMOGRAPHY

The diagnostic aids described above are useful and widespread not only in science, but also in public and private clinics. In several countries, their use is reimbursed by health insurances.

However, due to the low resolution (OCT) and penetration depth (RCM), there remain limitations to overcome for their even more widespread use. Recently, a new device line-field confocal OCT was developed. This is a new technique with cellular resolution and penetration depth into the middle dermis, which can determine not only the morphology of many tumors but also the depth of invasion.[49-51]

Line-field confocal optical coherence tomography (LC-OCT) was developed by a French start-up company (DAMAE-medical, Paris, France) and aims to combine the advantages of the above two diagnostic methods. LC- OCT consists of a two-beam interference microscope that uses a continuous-spectrum laser source of 800 nm wavelength and employs a line-scan camera as the photodetector.

The radiation of the laser corresponds to laser classification 1M according to EN 60825-1. The principle is based on a time-domain OCT (TD-OCT), which creates multiple A-scans of the skin surface in parallel at a depth of 500µm for the acquisition of B-scans while continuously refocusing. The parallel acquired A-scans enable dynamic fast focusing of the B-images with high lateral (1.3 µm) and axial (1.1 µm) resolution and simultaneously high frame rate.

The device system consists of an LC-OCT probe and a stable device trolley, his PC with monitor and keyboard, a shelf and a laser unit. Immersion oil is applied between skin and probe to reduce reflections and balance refractive index. No contrast agent is required. Contrast in grayscale images is created by differences in the strength of reflections of the skin's natural chromophores, such as keratin and melanin. Again, pigmented cells appear very bright compared to the dark, water-rich cytoplasm due to the different refractive indices of air and water.[49,52,53]

The latest prototype is also equipped with a camera, which enables live navigation in the examined area. The user moves the probe over the area to be inspected.

The LC-OCT creates these black-and-white images in 3 modi in real time: vertically (*en-coupe*) as in OCT and histology, horizontally (*en-face*) as in RCM and dermoscopy, and as a 3D image. Videos can also be stored. The high image acquisition speed is 10s for a 2-D image and 15s for a 3-D stack image. This means that the images can be recorded and evaluated on the screen at the same time. To switch between the individual modes and to record the videos, corresponding keys on the measuring head are activated. The individual image section generated in this way has a size of 1.2×0.5 mm in the vertical and horizontal view.

Acquired images can be exported quickly, and storage to an external device is possible in TIFF, DICOM, or JPEG formats.

The deepLive™ machine is CE certified for clinical use, and has recently become commercially available; during the course of this study project, it was only being used in few highly selected university centers around the world as part of experimental research.

LC-OCT images, like OCT images, can be interpreted with basic histological knowledge, especially in vertical sections. A broad stratum corneum interrupted by sweat gland ducts is visible. Individual keratinocytes are also visible in the granule/spinous layer as they increase in size. The transition from dermis to epidermis varies in brightness depending on the phototype. In the dermis, lighter collagen fibers are found, along with darker blood vessels where motile blood cells are found. Comparing to histological sections is very intuitive, especially for longitudinal images, while transverse images take some practice to interpret, but are similar to RCM images. (Figure 4)

Up to now, main fields of application of LC-OCT seem to be epithelial skin cancers and their precursors, especially basal cell carcinomas, actinic keratoses, Bowen's disease and squamous cell carcinomas. Furthermore, inflammatory, infectious and parasitic skin diseases can be visualized, imaged and monitored with LC-OCT. The number of scientific publications on this topic has significantly increased in few years, shedding light on the enormous potential of this device in the field of dermatology.[5,49,50,52-80]

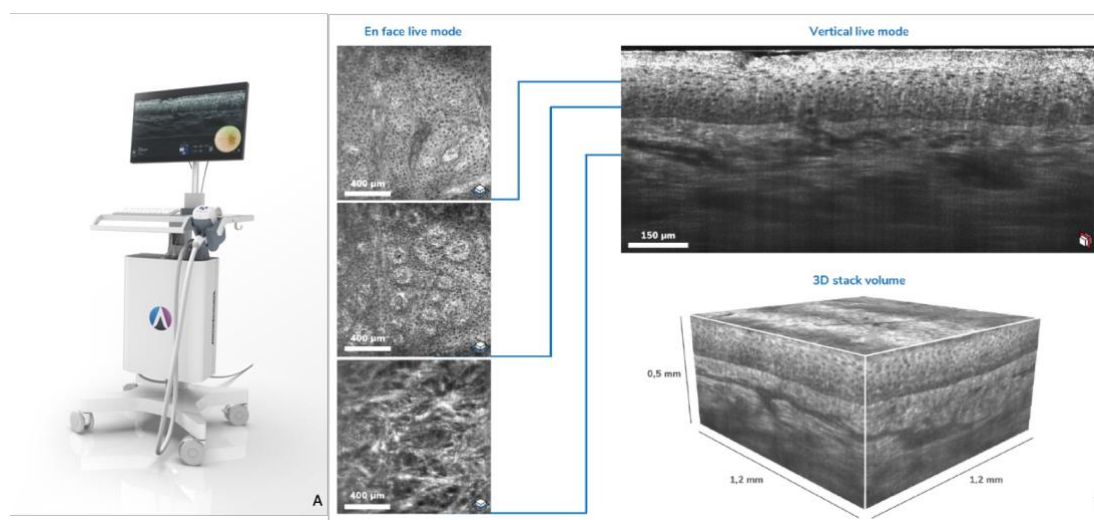


Figure 4. Commercially available LC-OCT device deepLive™, DAMAE Medical, Paris, France (A) and correlation of LC-OCT images to the corresponding skin layers (B) in different acquisition modes.

DERMATOFLUOROSCOPY

For several years, research tried to analyze the differences in melanin fluorescence between malignant and benign lesions. Magnosco (Berlin, Germany) has developed DermaFC, a compact device that has shown a sensitivity of 89.1% and a specificity of 44.8% in melanoma diagnosis in the pilot study. Further, larger studies found a highly significant difference between the diagnostic scores of different skin tumors, with the highest sensitivity for MM (92.1 %), but also for pigmented BCCs. The higher sensitivity correlated with older age, skin tanning and freckles during childhood.[81-84]

The system is based on following principles: stepwise two-photon absorption selectively excites melanin and visualizes its fluorescence. Nanosecond laser pulses at 800 nm are focused in the epidermis; the corresponding melanin fluorescence is detected in the range of approximately 385 - 785 nm. This can suppress the normal autofluorescence of the natural endogenous fluorophores in the cells. The process is called fluoroscopy and is then used selectively to distinguish benign from malignant melanocytic lesions, each of which has characteristic spectral differences. For example, a "red shift" in the fluorescence spectrum has been described in the malignant transformation of melanocytic lesions, as it corresponds to "degeneration". A plastic ring, covered by a transparent adhesive film, is stuck on the lesion to be examined. To avoid interference, the surrounding area is covered with dark paper (alternatively, the examination should be performed in a dark room). A mobile arm with the device head is then connected and the lesion is photographed through the integrated camera. The area to be scanned is selected on the image folder and analyzed point by point with a step size of 200 μm . Automated data analysis should then evaluate the extent of spectral shift, frequency, and spatial distribution. The algorithm was also extended by the neural self-learning method. The result is a numerical score with a cut-off of 28 for melanoma. The methodology is applied both in vivo and ex vivo (on formalin-fixed paraffin-embedded specimens). The in-vivo

examination is painless and harmless, but may take more than 5 minutes. Also in this case, the device is applied for objectifiable support of clinical diagnosis.

OPTOACUSTIC IMAGING

Optoacoustic imaging (OAI) is a hybrid imaging modality that integrates the benefits of optical contrast and ultrasound detection. OAI is a non-invasive imaging method that relies on the thermoelastic expansion of tissue to detect acoustic signals utilizing an ultrasound transducer and a pulsed laser for illumination. Optoacoustic tomography employs a detector with a lower central frequency to gather signals from larger vascular structures at longer wavelengths, resulting in deeper penetration depths but poorer lateral resolution. Optoacoustic microscopy, a higher resolution configuration of OAI system, is suitable for imaging skin microvasculature using wideband ultrasound detector, allowing deeper imaging (about 2 mm) with high lateral resolution of 40 μm . Raster-Scanning Optoacoustic Mesoscopy (RSOM) can be used in research for evaluating epidermis thickness, vessel diameter in the dermis, capillary loop density in the dermal layers as well as functional data like oxygen saturation in skin microvasculature. Such data are useful for determining morphological and physiological differences between non-lesional and lesional skin areas of patients affected by atopic dermatitis, eczema or sclerosing diseases. Furthermore, this technique has been used in the non-invasive detection of melanoma metastases in sentinel lymph nodes (SNL). So far, few pilot studies are available, showing for example a concordance of 96.4% between OAI and Technetium-99-marked SLN basins.[85-88]

Main commercially available devices are produced by the company iThera (Munich, Germany). Two main machines are available for clinical research: MSOT and Raster-Scanning Optoacoustic Mesoscopy (RSOM). They mainly differ in resolution and the penetration depth. Where the MSOT has a penetration depth of up to 3 cm and a resolution up to 80 μm , the RSOM has a different way to generate the signals (raster-scanning) with a resolution up to 10 μm but a penetration depth of 2-3

mm. The MSOT Acuity Echo is used with a handheld detector similar to ultrasound and has a tunable laser source offering the range from 600 nm to 1300 nm thus it allows the detection of all types of endogenous or exogenous absorbers. An ultrasound for better orientation and super-positioning is included in this fully certified medical product. The RSOM is available in two different versions, one with an integrated box for mice meaning intended for animal testing only, while the second version comes with a handheld detector for both human and animal use. (Figure 5)



Figure 5. Commercially available MSOT Acuity Echo device, iThera GmbH, Munich, Germany.

NON-IMAGING TECHNIQUES

Innovative complementary methods to improve daily diagnostic routine also include non-imaging techniques, that yield a numerical rather than a morphologic representation of the skin lesions examined. The calculation of the score represents a recommendation for further action, such as excision of a lesion suspected of malignancy. In contrast to other techniques, the devices falling into this category require very short training; moreover, the numerical score is immediately comprehensible to the patient.

ELECTRICAL IMPEDANCE SPECTROSCOPY

Electrical impedance spectroscopy (EIS) has been proposed as non-operator dependent, objective tool to distinguish between benign and malignant lesions. It was evaluated in large multicenter studies,

which showed high sensitivity. Nevisense® (Scibase AB, Stockholm, Sweden) produces an easily portable device specialized in the risk assessment of melanocytic lesions. The device consists of a small touch screen monitor and a measuring head equipped with disposable electrodes. The examination is painless and takes a few seconds. The technology is based on the principle of different electrical skin impedance (resistance) in healthy skin, benign and malignant melanocytic lesions. In particular, through the frequencies used (1 kHz-2.5 MHz), the device should detect the different electrical properties that depend on the cell size, shape and orientation. The small spikes of the electrodes of the measuring head are pressed on the skin surface, thus a current pulse flows, which influences the configuration of the underlying cells. The measurement of the resulting values, represented as impedance curves, depends on the structure of the cells (regular or chaotic). An algorithm was developed in large studies to represent the degree of atypia and the probability of the presence of melanoma. The suspicious lesion is then analyzed and its impedance is compared to reference values of healthy skin. This results in a risk value calculated between 0 and 10. From a score of 4, excision can be recommended, although the positive predictive value of the abnormal numbers increases between 9% (score 4) and a maximum of 64% (score 10). A pilot study showed a very high sensitivity of 97%, with a specificity of 34% in melanoma diagnosis. The device is mainly intended for the differentiation of melanocytic tumors, even though also NMSC was investigated. The application of the device is not reliable on certain areas (scalp, palmo-plantar regions, elbows and knees) and on ulcerations or wounds. This diagnostic method is suitable for rapid support of clinical and dermatoscopic diagnosis of melanocytic lesions.[89] (Figure 6)



Figure 6. Commercially available Nevisense EIS device, Scibase AB, Stockholm, Sweden

Raman spectroscopy (RS) is an optical technique based on the analysis of inelastic monochromatic light scattering in materials, where incident light converts energy into molecular vibrations. Skin investigation is performed by applying a handheld probe emitting a 758 nm laser to the skin surface; following the exposure of monochromatic laser light, the energy of the photons is transferred to the molecules of the sample, subsequently originating the scattered light. The resulting inelastic light scattering is plotted on the spectrum to obtain the molecular fingerprint of the tissue. The amount of the transferred energy depends on the masses of the atoms of the particular molecule and the chemical bonds between them; therefore, the Raman spectrum is highly molecule specific. As a consequence, RS allows the analysis the molecular composition of skin including water content, natural moisturizing factor, ceramides, and cholesterol. This can be particularly useful for monitoring the changes in molecular concentrations of skin compounds following the application of drugs or topical cosmetics, especially in diseases such as atopic dermatitis. Concerning skin cancer, RS has been used in small pilot studies showing a sensitivity of 95-99% and a specificity of 15-54%, distinguishing between benign and malignant skin lesions (melanoma and non-melanoma skin cancer – NMSC).[11,90-94] (Figure 7)

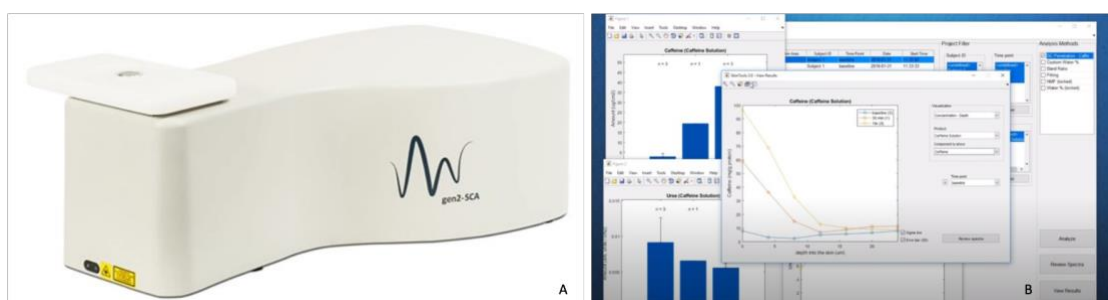


Figure 7. Commercially available gen2-sca confocal Raman system for skin analysis (A) and screenshot of its monitor with the SkinTools3 software, RiverD international B.V., Rotterdam, the Netherlands

SKIN CANCER

Skin cancer is one of the most common malignancies in the general population. The incidence of skin cancer is increasing at an alarming rate each year. Increased morbidity and mortality are also reflected

in increased treatment costs. Skin cancers are mainly divided into two classes: MM and NMSC, and their precursors.

MM is the most invasive type and presents with high morbidity and mortality if undetected in its early stages. NMSC (mainly AK, BD, SCC, BCC) mainly appears on sun damaged skin on elderly patients of light skin phototype.

BCC is a malignant, slow-growing, locally destructive epithelial skin tumor originating from cells of the basal cell layer and/or the outer root sheath of the hair follicle. They often invade tissue irregularly with finger-like projections. Metastasis is extremely rare, but morbidity is determined by local tissue invasion and destruction, which can reach frightening proportions (ulcus terebrans, ulcus rodens). About 80% of BCC are localized in the head and neck and primarily affect light-skinned men and women (caucasians). BCC is the most common malignancy in the general central European population. Treatment of BCC is ideally based on clinical evaluation, but it is usually not possible to accurately estimate the extent of tumor width and depth. Because tumor growth often exceeds clinically recognizable rates, the first excision is often not complete, especially for large tumors.

After histologic confirmation of the diagnosis, treatment is performed according to location, extent, and subtype. BCCs are usually surgically removed, but superficial forms can also be treated with cryosurgery, laser therapy, topical medications, or photodynamic therapy (PDT). In inoperable forms, radiotherapy, electrochemotherapy, and systemic therapy with inhibitors of the ptch1 signaling pathway can be considered.[95-97]

For BCCs in the facial area, surgical treatment can have a very devastating effect and often requires reconstruction with plastic. Special histological preparation methods for microscopically controlled surgery are used to protect and ensure optimized recurrence-free wound closure. These differ in incision and assessment and are intended to ensure the integrity of surgical tumor removal. All techniques mark the removed tissue and create a sketch to ensure correct topographic orientation.

The preferred method is to display gap-free cutout edges. These include the Tübingen cake and the Munich method. In the Munich method, specimens are not fixed in formalin but are moistened in saline. Transverse cryosections are taken from the tissue and stained with hematoxylin-eosin (H&E) to examine the outer margin and base. Once the excision is complete, wound closure can be performed on the same day or the next day. If the tumor remains, more incisions are made in the affected area. This procedure is repeated until the tumor is safely and completely removed. Chance of recurrence is low compared to conventional surgery.[98-100]

Cutaneous SCC also occurs primarily in sun-exposed areas and is the second most common skin tumor after BCC. The highest incidence occurs on the face and scalp in those over 50 years of age. Growth factors (such as hair color, family history, degree of sunburn, and exposure to sunlight, especially ultraviolet light) play an important role in its development. Moderate AK with local therapy such as topical agents (imiquimod, 5-fluorouracil, diclofenac, tirbanibuline, ...), cryotherapy or PDT, advanced SCC should be surgically removed. Metastatic potential is closely related to tumor size (>2 cm) and deep growth into the underlying tissue. Metastatic and inoperable tumors usually have a poor prognosis and can be treated with radiotherapy, conventional chemotherapy (cisplatin, 5-fluorouracil), or immunotherapy (anti-PD1 antibodies).[101]

Malignant melanoma is responsible for 90% of all malignant skin tumor mortality, and its frequency has increased significantly in recent decades. This tumor also primarily affects the Celtic skin type, and acute and intermittent exposure to intense ultraviolet light is an important risk factor. However, the risk of metastasis increases significantly with the thickness of the histologic tumor, and despite chemotherapy, immunotherapy, or radiation therapy, the tumor is usually fatal. New systemic targeted therapies (BRAF and MEK inhibitors) and immunotherapies (PD1 and CTLA4 immune checkpoint inhibitors) have brought much hope for treatment. [102,103]

AIMS OF THE PROJECT

The main aim of this project was to explore the first clinical applications of the novel device LC-OCT in the field of dermatology, specially focusing on the standardization of the technique, the analysis of cytological and architectural correlations with histopathology, the development of specific image acquisition protocols, standard nomenclature, the identification of main artefacts and confounders and the comparison of LC-OCT with other more established non-invasive diagnostic techniques. The project was articulated in several sub-studies, each centered on distinct types of skin lesions. In detail, main aims of this project were:

- to assess standard image acquisition protocols with LC-OCT, assessing technical limitations and pitfalls
- to investigate healthy skin at different anatomical locations and individually evaluate skin layers and adnexa
- to preliminarily define main imaging criteria and histological correlations of AK, BD and SCC using LC-OCT; we moreover aimed to define criteria for an LC-OCT grading of AKs based on the PRO classification and to correlate it with its histological counterpart, as well as to evaluate the interobserver agreement for this LC-OCT based PRO classification.
- to describe main cytological and architectural features of BCC seen in LC-OCT, to evaluate the main LC-OCT criteria for the diagnosis and subtyping of BCC compared with histopathology, OCT and RCM; moreover, we aimed to investigate the accuracy of LC-OCT in diagnosing clinically unsure cases of BCC compared to dermoscopy alone and in distinguishing between superficial BCCs and other BCC subtypes.
- to evaluate LC-OCT for the discrimination between nevi and melanomas, in comparison to RCM
- to verify the potential applications of LC-OCT outside dermato-oncology, in particular in the diagnosis of parasitic infestations and inflammatory skin diseases.

METHODOLOGY

The project was articulated in several sub studies focused on research in the correlation between LC-OCT imaging criteria and their histopathological counterparts, as well as the corresponding dermoscopy, OCT and RCM criteria. (Table 1) Particular attention was furthermore given to the application of the technology in daily clinical practice to provide patient triage, to the assessment of diagnostic confidence and interobserver agreement. Clinical, dermoscopic and LC-OCT images of seven hundred skin cancerous and inflammatory lesions and healthy skin areas were prospectively collected; morphological criteria based on the histological and established imaging nomenclature were assessed, compared to the corresponding hematoxylin & eosin (H&E) stained histological slides as well as OCT and RCM images when available. Diagnostic confidence and imaging quality were evaluated. Dermoscopic images were collected with the FotoFinder system (FotoFinder GmbH, Bad Birnbach, Germany); LC-OCT images were acquired with evolving prototypes of LC-OCT (deepLive™, DAMAE medical, Paris, France). Further images were collected with the commercially distributed RCM devices (VivaScope® 1500 and VivaScope® 1500/3000 Combo, Mavig GmbH, Munich, Germany) and OCT device VivoSight® (Michelson Diagnostics Ltd., Maidstone, Kent, UK). The statistical analysis was chosen based on the sub-study objectives; we used descriptive statistics, weighted Cohen's kappa coefficients with linear weights, as well as univariate and multivariable multinomial logistic regressions. All statistical analyses were conducted using R (version 3.6.0, 2019, R Foundation for Statistical Computing). The protocol was reviewed and approved by the local review board (17-699), written informed consent was provided by all patients.

Device	Sections	Field of view	Resolution	Penetration depth	Time needed	Interpretation
RCM	horizontal	500x500 µm, mosaic 8x8 mm	1-3 µm	200-250 µm	minutes	Dermoscopy like
OCT	vertical (horizontal, 3D)	6x6x1.5 mm	7.5-10 µm	1.5 mm	seconds	Ultrasound like
LC-OCT	horizontal, vertical, 3D	1.2x1.2x0.5 mm	1.1-1.3 µm	500 µm	seconds	Histology like

Table 1. Diagnostic devices employed for this project with their main features

I. HEALTHY SKIN

Adapted from the original article: “Ruini C, et al., Line-field confocal optical coherence tomography-Practical applications in dermatology and comparison with established imaging methods. *Skin Res Technol.* 2021 May;27(3):340-352.”

Background

No systematic, prospective studies on LC-OCT investigation of healthy skin or malignant skin lesions had been published at the time of the study.

Aim

The first part of our project was focused on the preliminary applications of LC-OCT in the in-vivo analysis of healthy skin, adnexa and most common skin cancers compared to histology, OCT and RCM. Acquisitions protocols were developed with attention to avoiding artefacts.

Methods

Healthy skin in different body areas (right cheek, right volar forearm and right middle fingertip) were investigated with LC-OCT (en-face, vertical and 3D), OCT (vertical and dynamic en-face), RCM (VivaBlock® and VivaStack). An adhesive ring was used to mark the measuring position. Following skin layers were individually evaluated: stratum corneum (SC), epidermis (EP), dermo-epidermal junction (DEJ), papillary dermis (PD) for all three methods, and reticular dermis (RD) only in OCT. Healthy skin at different body sites and exemplary skin lesions (BCC, MM, AK) were examined using dermoscopy, RCM, OCT and LC-OCT. Standard features for RCM and OCT and comparable features for LC-OCT were analyzed.

Results

The SC in vertical mode is composed of corneocytes. Detached super-reflective scales may be visible. Erosion appears as dark, irregular surface discontinuities. Keratinocytes are slightly dark polygonal cells with light contours. They decrease in size from SC to stratum granulosum (SG) to stratum spinosum (SS). Hair follicles appear as longitudinal dark shadows, sometimes with light shafts. The DEJ is a thin dark line composed of a layer of small basal cells. It separates the well-delineated epidermis from the papillary dermis, the latter appearing hyperreflective. Blood vessels are represented as hyporeflective linear and tortuous structures.

In frontal mode, hyperreflective single corneocytes with 10–30 μm on the surface appear like in RCM. The keratinocytes that make up the EP are polygonal cells with dark nuclei, arranged in a honeycomb-shaped pattern. The more pigmented keratinocytes appear brighter and produce a cobblestone pattern. Skin folds are sharply defined, non-refractive, linear grooves between groups of keratinocytes. The follicle is also a highly identifiable concentric circular structure with a dark circular central opening surrounded by a stratification of dark epithelial cells. The DEJ in normal skin has a regular annular structure consisting of a dark round to oval dermal papilla centered on a capillary loop, depending on the cross-section of the dermal papilla. These correspond to the horn papillae displayed on the RCM.

Hyperreflective collagen fibers and bundles and hyporeflective linear and spherical vessels predominate in the papillary dermis.

As usual in appendage-rich skin, the surfaces of the (thin) SC, EP, and DEJ in vertical mode appear slightly wavy. Sebaceous glands appear as spherical, light-colored structures attached to the epidermis and may have openings to hair follicles. Several bright, rounded, homogeneous comedones filled with amorphous material are usually visible. Demodex mites as skin symbionts appear in the hair follicles as hyperreflective globules in vertical mode and as large clustered hyperreflective structures in frontal mode. The DEJ is slightly flattened in the elderly skin; the dermis is lighter in young subjects than in older subjects, possibly due to a more compact structure of collagen fibers.

Because the skin appears thinner, it appears darker in older skin compared to younger, especially in frontal mode. (Figure 8)

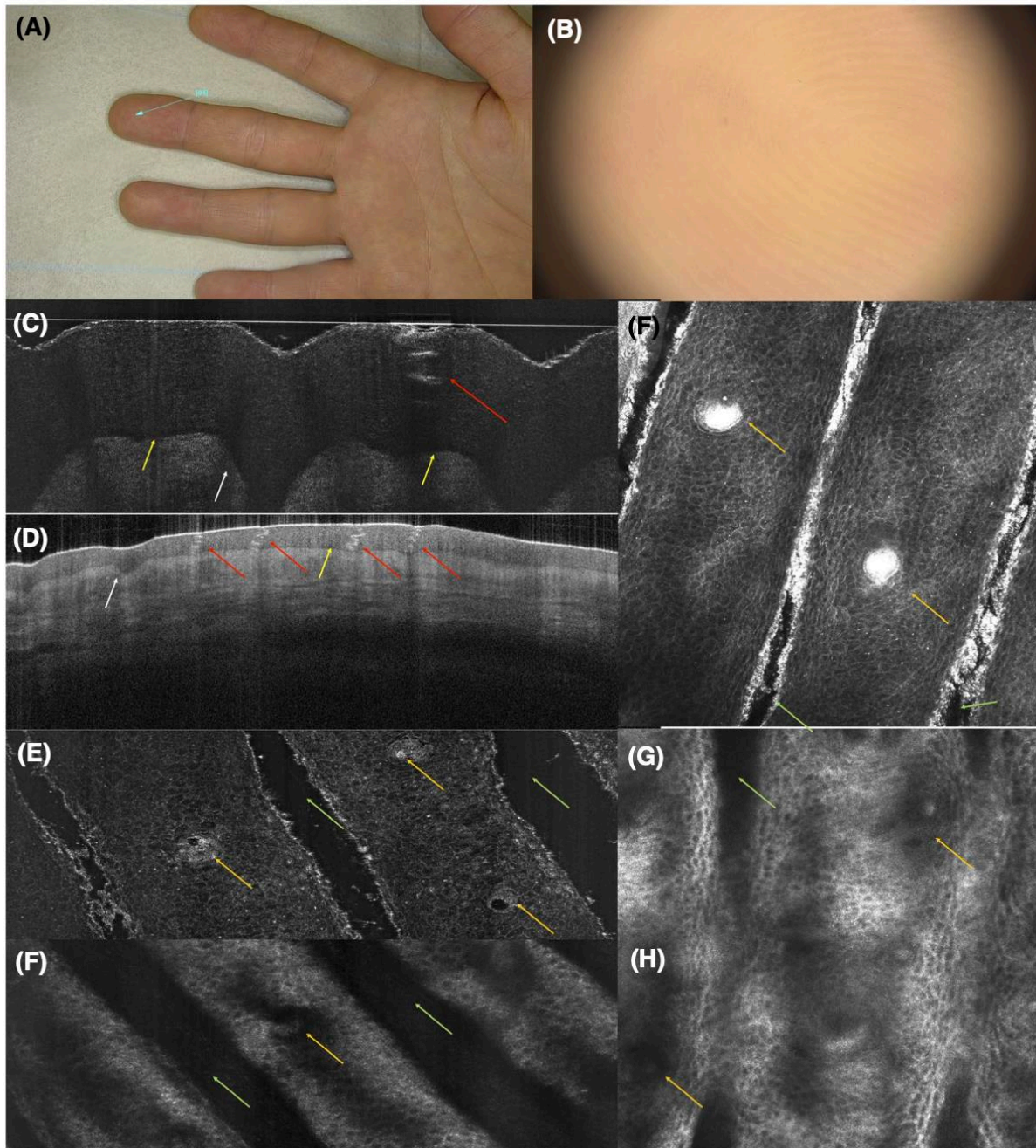


Figure 8. Healthy skin on the fingertip of the index finger acquired with LC-OCT, RCM and OCT. Clinical (A) and dermoscopy (B) picture indicate the scanned area. In vertical LC-OCT (C) and OCT (D) images, the typical thickened SC is visible, with the characteristic wavy pattern of the surface and bright entral signal. Hyperreflective, coiled eccrine ducts can be seen in the epidermis (red arrows). A darker suprabasal line may correspond to the stratum lucidum (yellow arrows). The DEJ is bright and follows the undulating pattern (white arrows). The dermis cannot be effectively assessed

at the fingertip due to the increased epidermal thickness and therefore limited penetration depth. In en-face mode (LC-OCT: C,E; RCM: D, F), skin folds are very prominent dark furrows separating islands of polygonal keratinocytes (green arrows). Roundish hyperreflective structures with a dark centre are regularly distributed among ridges of the skin marks and correspond to eccrine sweat ducts (orange arrows in C, E in LC-OCT images and D, F, G, H in RCM images)

LC-OCT is able to visualize cytological and architectural components of healthy skin and main adnexa in greyscale. The predominant morphological pattern of healthy skin in LC-OCT seems to be consistent with the respective OCT when in vertical mode, and RCM features when in horizontal mode.

II. FIELD CANCERIZATION

Adapted from the original articles:

Ila. “Ruini C, et al. Line-field confocal optical coherence tomography for the in vivo real-time diagnosis of different stages of keratinocyte skin cancer: a preliminary study. *J Eur Acad Dermatol Venereol*. 2021 Dec;35(12):2388-2397”

Ilb. “Ruini C, et al. In-Vivo LC-OCT Evaluation of the Downward Proliferation Pattern of Keratinocytes in Actinic Keratosis in Comparison with Histology: First Impressions from a Pilot Study. *Cancers (Basel)*. 2021 Jun 8;13(12):2856.”

Background

Treatment of keratinocyte carcinoma (KC) is strictly dependent on its differentiation and invasiveness. AK and BD can mostly benefit of local treatments, while SCC requires a surgical excision. It is moreover known that AKs can progress to invasive SCC; the histological PRO grading of AKs, based on the growth pattern of basal keratinocytes, relates to their progression risk. The PRO classification (named after "protruding") classifies the growth pattern of basal keratinocytes into three categories (PRO I-III). In stage I, several atypical keratinocytes are crowded over the basal layer; in stage II, basal protrusions into the upper dermis form small bunches of atypical keratinocytes. In stage III, larger nests of atypical keratinocytes encompass digitiform protrusion into the stratum papillare.

In order to prevent the transformation from subclinical dysplasia to invasive SCC and to lower the social and economic burden of the disease, early detection and treatment are essential. Dermoscopy and non-invasive diagnostic techniques such as LC-OCT can support live diagnosis and avoid unnecessary biopsies.

Aims

The first sub-study aimed to define standard LC-OCT diagnostic criteria for the spectrum of KC based on the histologic correlations of the different entities, from AK to BD to SCC. Since some AK can progress to SCC, the second sub-study aimed to define criteria for the bedside LC-OCT grading of AKs based on the PRO classification, including interobserver agreement, and to correlate it with its histological counterpart. A preliminary comparison of LC-OCT images with OCT and RCM was also carried out to exemplarily point out the differences, strengths and limitations of the methods.

Methods

Seventy-three histopathologically confirmed lesions (46 AK, 11 BD and 16 SCC) imaged with dermoscopy and LC-OCT were collected, 10 AK, 5 BD and 5 SCC were additionally investigated with OCT and RCM. Detailed criteria are displayed in the table. The level of diagnostic confidence was defined on a three- point scale from 0% to 100%. Instrument and acquisition procedures were described above and elsewhere.[69,71] For descriptive statistics, mean values with 95% CI were calculated for numeric data, while absolute numbers with percentage portion were given for nominal data. Univariate multinomial logistic regressions were performed for each individual feature, odds ratios (OR) with corresponding P-values were also calculated. All predictors with significant OR were included in a multivariable multinomial logistic regression analysis. In the second study, fifty AKs were imaged by LC-OCT and biopsied for histopathology. Histological PRO grading was assessed by expert consensus, while two groups of raters separately performed his LC-OCT grading on vertical sections. Weighted Cohen's kappa coefficients with linear weights were used to calculate correlations between LC-OCT and histopathological PRO scores, including CI. Correlations were also estimated in different subgroups (PRO I and II/III). A p-value less than 0.05 was considered significant.

Results

The use of LC-OCT brought to an improvement of diagnostic confidence of 24.7% compared to clinical examination and dermoscopy alone. The main LC-OCT features of vertical mode AK were: hyperkeratosis with compact parakeratosis (95.7% and 67.1%, respectively), scaling (67.4%), epidermal hypertrophy (68.2%), and keratinocyte atypia (95.5%). It has not always been possible to accurately distinguish between epidermal layers. BD was characterized by prominent keratinocyte atypia encompassing all epidermal layers (100%) but was accompanied by well-defined DEJ (80%). Infiltrating SCC showed hyperkeratosis (100%), ulceration (68.8%), and destruction of SC (100%), destroyed or invisible DEJ (81.2%). Vasodilation/neovascularization (78.6%) and collagen alterations (75%) were found. (Figure 9)

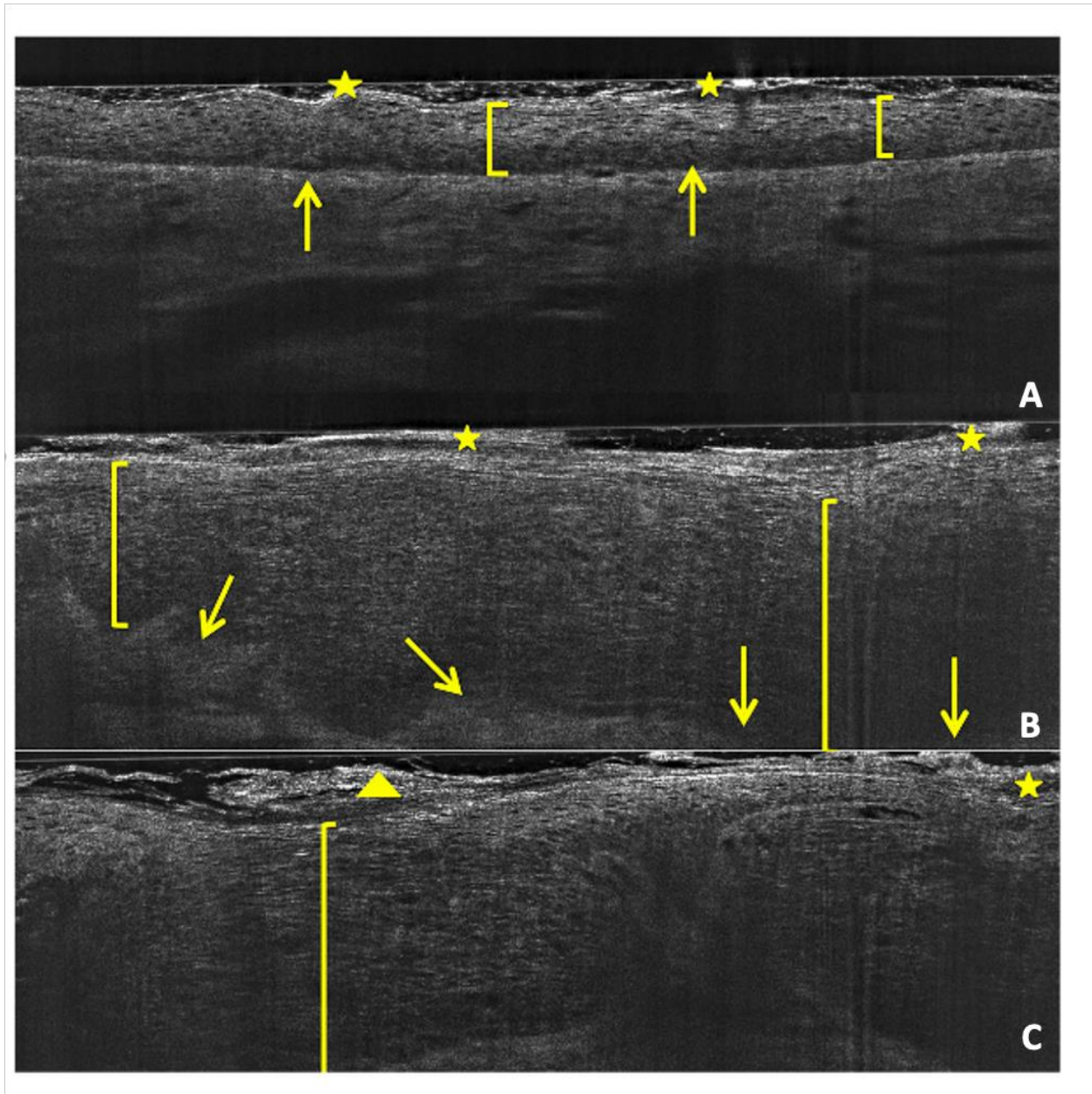


Figure 9. LC-OCT images of progressive stages of KC. AK of the face (A), BD of the back (B), scalp SCC (C). Following features are seen: hyperkeratosis and parakeratosis (star), atypical keratinocytes (bracket); preserved DEJ in (A) and (B) but not in (C), where strands of atypical keratinocytes infiltrating the dermis are seen (bracket) together with a thick, hyperkeratotic crust (triangle).

The average epidermal thickness was 126.2 microns in AK, 168.5 microns in BD and 232 microns in SCC. A univariate multinomial logistic regression model was run to identify key LC-OCT patterns that could distinguish different entities (AK, BD, and SCC). The following features helped distinguish SCC from AK: increased epidermal thickness ($P < 0.001$), ulceration ($P < 0.001$), keratin plugs ($P < 0.001$), acantholysis ($P < 0.05$), bright particles in the epidermis ($P < 0.01$), flattened rete ridges ($P < 0.001$).

0.05), not visible/interrupted DEJ ($P < 0.001$) and bright particles in the dermis ($P < 0.05$). Ulceration ($P < 0.05$) and bright particles in the epidermis ($P < 0.05$) could analogously help differentiate a SCC from a BD, while the OR for BD well-defined DEJ ($P < 0.01$) and bowenoid pattern ($P < 0.01$) was significantly higher for BD compared to SCC. The bowenoid pattern was the distinguishing feature of BD compared to AKs ($P < 0.001$). (Table 2, 3)

Features	All subtypes		Actinic keratosis		Bowen's disease		Invasive squamous cell carcinoma	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Epidermal thickness	155.8	134.0, 177.5	126.2	102.9, 149.5	168.5	107.1, 229.8	232.0	182.3, 281.7
	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>
Epidermis								
Ulceration	23.9	17/71	6.8	3/44	27.3	3/11	68.8	11/16
Disruption of stratum corneum	82.2	60/73	71.7	33/46	100.0	11/11	100.0	16/16
Hyperkeratosis	97.3	71/73	95.7	44/46	100.0	11/11	100	16/16
Compact parakeratosis	83.6	61/73	67.1	35/46	100.0	11/11	93.8	15/16
Scales	78.1	57/73	67.4	31/46	90.9	10/11	100.0	16/16
Keratin plugs	50.7	37/73	39.1	18/46	54.5	6/11	81.2	13/16
Thinning of the epidermis	28.2	20/71	40.9	18/44	18.2	2/11	0.0	0/16
Broadened/acanthotic epidermis	78.9	56/71	68.2	30/44	100.0	11/11	93.8	15/16
Suprabasal atypical keratinocytes	71.2	52/73	54.3	25/46	100.0	11/11	100.0	16/16
Basal atypical keratinocytes	94.5	69/71	95.5	42/44	100.0	11/11	100.0	16/16
Bowenoid pattern	27.8	20/72	15.2	7/46	90.0	9/10	25.0	4/16
Acantholysis	23.2	16/69	15.9	7/44	20.0	2/10	46.7	7/15
Pyknotic cells	43.1	31/72	32.6	15/46	20.0	5/10	68.8	11/16
Atypical honeycombed pattern	97.2	70/72	95.7	44/46	100.0	10/10	100.0	16/16
Bright particles in the epidermis	31.4	22/70	25.0	11/44	10.0	1/10	62.5	10/16
Dendritic cells	5.7	4/70	4.5	2/44	20.0	2/10	0	0/16
Flattened rete ridges	56.5	39/69	65.1	28/43	5.5	6/11	33.3	5/15
Dermo-epidermal junction								
Well defined	68.1	49/72	82.6	38/46	80.0	8/10	18.8	3/16
Not visible/interrupted	28.8	21/73	15.2	7/46	36.4	4/11	81.2	13/16
Dermis								
Dilated vessels/neoangiogenesis	65.2	43/66	59.5	25/42	70.0	7/10	78.6	11/14
Glomerular vessels	13.6	9/66	9.5	4/42	20.0	2/10	21.4	3/14
Bright particles in the dermis	18.8	13/69	11.4	5/44	20.0	2/10	40.0	6/15
Elastosis/collagen alterations	61.9	39/63	56.1	23/41	70.0	7/10	75.0	9/12

CI, confidence interval; %, per cent (relative frequency); *n*, number (absolute frequency).

Table 2. Relative and absolute frequencies for LC-OCT features of AK, BD and SCC

Feature LC-OCT	Subtype	OR	P
Epidermal thickness	2 1	1.01	0.13
	3 1	1.01	$P < 0.001^{***}$
	3 2	1.01	0.10
Ulceration	2 1	5.12	0.07050
	3 1	30.06	$P < 0.001^{***}$
	3 2	5.87	$P < 0.05^*$
Disruption of stratum corneum	2 1	5548.60	0.81
	3 1	9400.90	0.81
	3 2	15.70	0.98
Hyperkeratosis	2 1	978.45	0.88
	3 1	896.47	0.85
	3 2	12.36	0.98
Compact parakeratosis	2 1	2212.53	0.76
	3 1	4.72	0.15
	3 2	0.00	0.84
Scales	2 1	4.80	0.15
	3 1	8192.20	0.78
	3 2	1509.60	0.81
Keratin plugs	2 1	1.87	0.36
	3 1	6.74	$P < 0.01^{**}$
	3 2	3.61	0.15
Thinning of the epidermis	2 1	0.32	0.18
	3 1	0.00	0.80
	3 2	0.00	0.85
Broadened/acanthotic epidermis	2 1	5689.80	0.80
	3 1	7.00	0.07
	3 2	0.00	0.99
Suprabasal atypical keratinocytes	2 1	12044.20	0.79
	3 1	20060.40	0.80
	3 2	12.60	0.99
Basal atypical keratinocytes	2 1	923.79	0.87
	3 1	867.28	0.84
	3 2	12.87	0.99
Bowenoid pattern	2 1	50.14	$P < 0.001^{***}$
	3 1	1.86	0.38
	3 2	0.04	$P < 0.05^{**}$
Acantholysis	2 1	1.32	0.75
	3 1	4.63	$P < 0.05^*$
	3 2	3.50	0.18
Pyknotic cells	2 1	2.08	0.30
	3 1	4.55	$P < 0.05^*$
	3 2	2.20	0.34
Atypical honeycombed pattern	2 1	1687.40	0.90
	3 1	3545.80	0.91
	3 2	12.4	0.98
Bright particles in the epidermis	2 1	0.33	0.32
	3 1	5.00	$P < 0.01^{**}$
	3 2	15.00	$P < 0.05^*$
Dendritic cells	2 1	5.250	0.12
	3 1	0.00	0.88
	3 2	0.00	0.84

Feature LC-OCT	Subtype	OR	P
Flattened rete ridges	2 1	0.64	0.52
	3 1	0.27	$P < 0.05^*$
	3 2	0.42	0.28
Well-defined DEJ	2 1	0.84	0.85
	3 1	0.049	$P < 0.001^{***}$
	3 2	0.058	$P < 0.01^{**}$
Not visible/interrupted DEJ	2 1	3.18	0.12
	3 1	9.28	$P < 0.001^{***}$
	3 2	2.92	0.19
Dilated vessels/neoangiogenesis	2 1	1.59	0.54
	3 1	2.49	0.21
	3 2	1.57	0.63
Glomerular vessels	2 1	2.38	0.36
	3 1	2.59	0.26
	3 2	1.09	0.93
Bright particles in the dermis	2 1	1.95	0.47
	3 1	5.20	$P < 0.05^*$
	3 2	2.67	0.30
Elastosis/collagen alterations	2 1	1.83	0.43
	3 1	2.35	0.25
	3 2	1.29	0.79

Table 3. Univariate multinomial logistic regression with OR and p-values of main LC-OCT features of AK, BD and SCC

In the second sub-study, agreement between LC-OCT and histological PRO grading was 75% for all lesions (weighted kappa 0.66, 95% CI 0.48-0.83, $p = < 0.001$) and 85.4% when comparing PRO I vs PRO II/III subgroups (weighted kappa 0.64, 95% CI 0.40-0.88, $p = < 0.001$). Interobserver agreement for LC-OCT was 90% (Cohen's kappa 0.84, 95% CI 0.71-0.91, $p = < 0.001$). (Figure 10, 11)

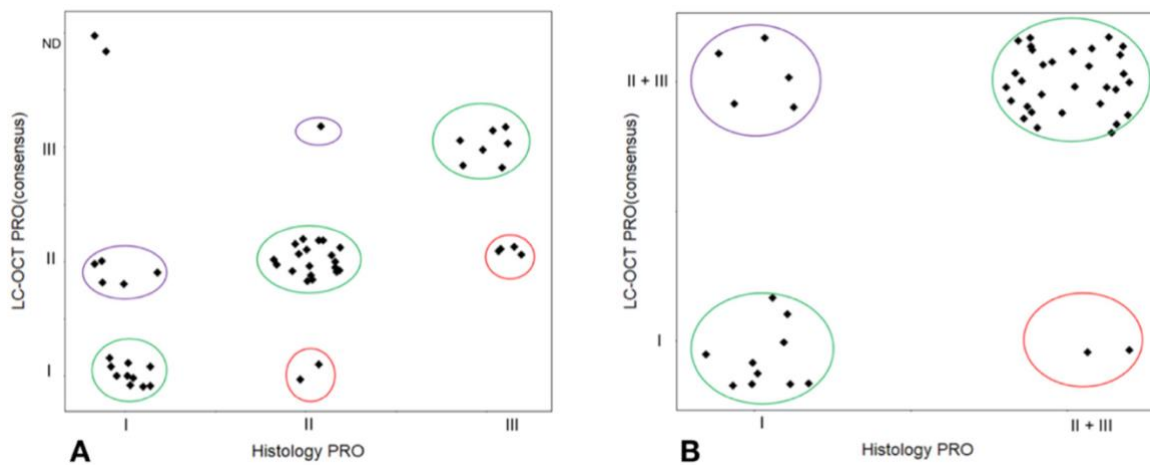


Figure 10. Overall (A) and subgroup (B) correlation between LC-OCT and histopathological PRO grading of AKs. ND: non definable.

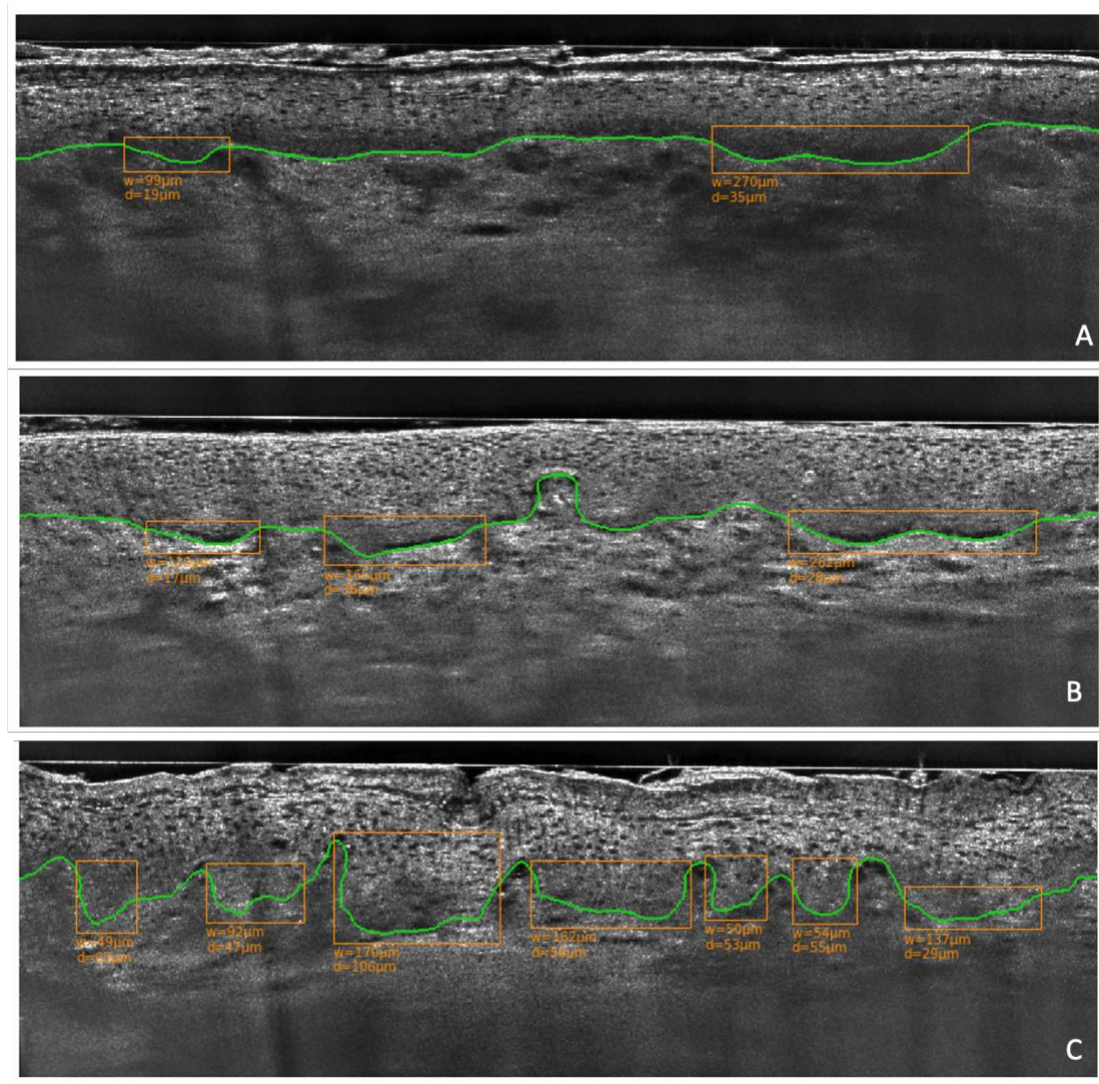


Figure 11. LC-OCT vertical acquisition of AK of different PRO grades, with A.I. based marking of the protrusion of the atypical keratinocytes into the dermis (A: PRO I, B: PRO II, C: PRO III).

Discussion

Real-time, non-invasive differentiation of field cancerization is of paramount importance in clinical practice as it enables efficient treatment planning.

Dermoscopy immediately helps the clinicians for an immediate evaluation, thanks to the intuitive progression model described by Zalaudek et al.[104] Our findings concerning dermoscopy were in line with the common literature on the topic: glomerular vessels were the most significant finding of BD, while starburst pattern, scales and ulceration were seen in SCCs.

Conventional OCT can detect common features of KC such as hyperkeratosis, epidermal disruption and loss of the DEJ. However, hyperkeratosis affects the imaging of underlying structures and an exact discrimination of the invasiveness is not always possible. More important, OCT does not allow a full evaluation of the cytology, due to its lower resolution.

On LC-OCT, hyperkeratosis is seen as hyperreflective multilayering of polygonal cells, sometimes with residual nuclei (parakeratosis). Atypical keratinocytes were polygonal structures with dark cytoplasm and light outlines, enlarged compared to those in healthy epidermis, and heterogeneous in size and shape. Cell stratification was intuitively comparable to the corresponding tissue sections. However, it has not always been easy to systematically distinguish between basal and suprabasal keratinocyte atypia. In this context, we found that differences in high-grade dysplasia (BD, SCC) are more easily discernible. Acantholysis was seen as darker spaces between epidermal keratinocytes. Fragmented (enriched) keratinocytes can also be detected. Epidermal thickness systematically and significantly increased with progression to invasive SCC. In the dermis, all lesion types in both vertical and horizontal cross-sections show increased angiogenesis and dislocation of collagen fibers, consistent with fibroelastosis (collagen response). Small hyperreflective particles, presumably corresponding to inflammatory infiltrates, can be visualized in both epidermal and dermal layers. LC-OCT could also visualize AK and its main histological correlates, as well as hints for their subtyping such as flattened rete-ridges and thin epidermis in atrophic AK. In BD, hypertrophied atypical keratinocytes form a thickened epidermis with rounded (“bowenoid”) contours in all epidermal layers can be seen. In BD and AK, the DEJ is well preserved and visible as a dark band separating the basal keratinocytes from the bright dermal collagen. In few cases (7 AK and 2 BD), SC thickness was increased, so that part of the DEJ was not visible, meaning that the epidermis and the DEJ cannot always be fully inspected, despite increased penetration depth. Similar to conventional OCT and RCM, possible strategies to partially overcome this limitation are to focus on the lesion borders and/or to remove thick keratinizing crusts; this can however lead to bleeding and inflammation, which

may compromise the examination. SCC is an infiltrating keratinizing tumor composed of large keratinocytes of various atypia that arises from the epidermis and spreads to the dermis. LC-OCT shows disorganized clumps of large polygonal cells with irregular contours. Rounded, bright and uniform structures were seen, corresponding to horn pearls. When visible, the dermis was infiltrated by tumor strands and masses and showed increased irregular angiogenesis (vasodilatation) and bright collagen bundles. In descriptive statistics, these findings were more frequent in his SCC than in AK and BD, but the model did not reach statistical significance, probably due to the small sample size.

A complementary point was to assess whether specific LC-OCT descriptors were significantly associated with different stages of keratinocyte cancer. We therefore included all predictors that showed a significant OR, in a multivariate multinomial logistic regression with bidirectional elimination and extrapolated some dermoscopic and LC-OCT features to quickly identify different types of KC. For example, continuous DEJ is a typical feature of AK and BD, but not seen in SCC. Disruption of the DEJ, marked collagen changes, and hyperreflective (inflammatory) particles within the dermis predominate in SCC. Various types of AK can also be detected better than dermoscopy.

Flattened reticular ridges are typical of hypertrophic AK, whereas thinned epidermis is seen in atrophic AK.

As seen in our second sub-study, LC-OCT is able to display atypical keratinocytes and their protrusion into the upper dermis as spikes, moreover it measures their thickness in comparison to the overlying epidermis. The LC-OCT PRO classification did not only relate to histology, but showed a high interobserver agreement, meaning it can be reproducible to estimate the potential of progression of AK to SCC in-vivo. Even though, there remain some limitations: very hyperkeratotic lesions are not fully evaluable. The agreement LC-OCT vs histology was worse in PRO I compared to PRO II/III, because of overestimation, as five histological PRO I AK were scored as imaging PRO II/III; such overestimation probably happened because of confounders (i.e. hair follicles and shafts), which appear as longitudinal structures. An advantage of LC-OCT is however the scanning of the whole

AK lesional area, and margins, in 3D and camera guided with multiple scans; histopathology has quite the reverse, with only one or few slides gained from each piece.

Concerning the study design, the small sample size requires further larger studies, while the experience of the user should be calibrated (in our study, all readers had good knowledge of both histopathology and diagnostic imaging techniques).

Conclusions

These results are preliminary and should serve as a guide for future studies on larger samples. Compared to OCT and RCM, LC-OCT is a unique combination of vertical and horizontal imaging with nearly cellular resolution, that facilitates histopathological correlation and reduces diagnostic pitfalls. Although its resolution is lower than in RCM, LC-OCT is able to show keratinocyte atypia; extensive hyperkeratosis may however make deeper layers of the epidermis inaccessible. On the contrary, only conventional OCT offers a dynamic blood flow mode. In the second sub-study, LC-OCT was capable of grading AK based on the in-vivo characterization of the basal growth pattern of keratinocytes, replicating the histological PRO classification in-vivo. A high interobserver agreement as well as a good correlation with histopathological slides were reported. Therefore, this classification system can be used to non-invasively estimate the malignant potential of AK and to address the lesion to the correct conservative or surgical treatment. We believe that the integration of clinical, dermoscopy and imaging data will improve treatment planning and follow-up in KC. In our experience, LC-OCT recognizes key features (keratinocyte morphology, architecture, and DEJ structure) and intuitive and reliable histopathological correlations, therefore facilitates the diagnosis of different stages of KC. Our results open new scenarios in the non-invasive characterization of field carcinogenesis.

III. BASAL CELL CARCINOMA

Adapted from the original articles:

IIIa. “Ruini C, et al. Line-field optical coherence tomography: in vivo diagnosis of basal cell carcinoma subtypes compared with histopathology. *Clin Exp Dermatol*. 2021 Dec;46(8):1471-1481”

IIIb. “Gust C,... Ruini C*, Sattler E*” Line-Field Confocal Optical Coherence Tomography Increases the Diagnostic Accuracy and Confidence for Basal Cell Carcinoma in Equivocal Lesions: A Prospective Study. *Cancers (Basel)*. 2022 Feb 21;14(4):1082. *shared authorship

Background

BCC is the most common skin cancer in the general population. Treatments vary from micrographic-controlled surgery to topical therapy depending on the subtype. Dermoscopy, RCM and OCT have entered clinical practice to optimize diagnosis and subtype-specific treatment. Namely, while nodular and fibrosing subtypes require surgical excision, superficial BCCs can be treated topically, with PDT or laser procedures. The new device LC-OCT enables imaging with high resolution and penetration depth, and is potentially suitable for the in-vivo detection of typical BCC structures in the different skin layers, allowing not only a differential diagnosis, but also a live subtyping and therefore therapeutic decision.

Aim

In the first sub-study, we defined the main LC-OCT criteria for the diagnosis and subtyping of BCC in comparison with histopathology, OCT and RCM. In the second sub-study, we investigated the accuracy of LC-OCT in diagnosing clinically unclear cases of skin lesions suspicious for BCC and in subtyping lesions, compared to dermoscopy alone. Moreover, we addressed diagnostic pitfalls.

Methods

All lesions were histologically confirmed. In the first sub-study, we examined 52 histopathologically confirmed BCCs; in the second sub-study, we prospectively enrolled 182 lesions of 154 patients, referred to our department because of suspected BCC. The frequency of morphological features, dermoscopic, LC-OCT and histomorphological patterns, image quality, diagnosis, BCC subtype, sensitivity, specificity and diagnostic confidence for LC-OCT and dermoscopy alone in comparison to histology and ROC curves were calculated. We used a linear regression to identify LC-OCT

features useful to differentiate between BCCs and non BCCs. A multinomial regression with stepwise variables selection to distinguish BCC subtypes was additionally performed.

Results

Nodular BCCs (nBCCs) were mainly characterized by atypical keratinocytes, altered DEJ, tumor nests in the dermis, hyporeflective clefting, prominent vascularisation and white hyperreflective stroma. Superficial BCCs (sBCCs) showed thickening of the epidermis by tumor nodules with clear association with the DEJ (bead chain pattern). (Figure 12) Fibrosing BCCs (fBCCs) were characterized by elongated hyporeflective tumor strands surrounded by pale collagen (shoal of fish pattern). The overall agreement of BCC subtype between LC-OCT and conventional histology was 90.4% (95% CI: 79.0, 96.8). The sensitivity and specificity of LC-OCT compared with histology were respectively: 96%,96% for nodular subtypes, 82%,100% for superficial forms, 100%,98% for fibrosing variants and 91%,95% for mixed, partly nodular partly superficial entities. (Figure 13) Multinomial logistic regression with stepwise selection of variables identified the following features as most useful for distinguishing BCC subtypes: thinned epidermis, atypical honeycomb pattern, prominent vessels/neoangiogenesis, shoal of fish pattern, string of pearls pattern and white hyperreflective stroma. In our second sub-study, diagnostic confidence was rated as “high” in only 48% of the lesions with dermoscopy alone compared to 70% with LC-OCT. LC-OCT showed a high sensitivity (98%) and specificity (80%) compared to histology, and even higher (100% sensitivity and 97% specificity) in the subgroup of lesions with high diagnostic confidence. Interobserver agreement was nearly perfect (95%). The combination of dermoscopy and LC-OCT reached a sensitivity of 100% and specificity of 81.2% in all cases and increased to sensitivity of 100% and specificity of 94.9% in cases with a high diagnostic confidence. The performance of LC-OCT was influenced by the image quality but not by the anatomical location of the lesion. Most specific morphological LC-OCT criteria in BCCs compared to non BCCs were: less defined DEJ,

hyporeflective tumor lobules, dark rim. Most relevant features of the subgroup of superficial BCCs (sBCCs) were: string of pearl pattern, absence of epidermal thinning. (Figure 14) (Table 4)

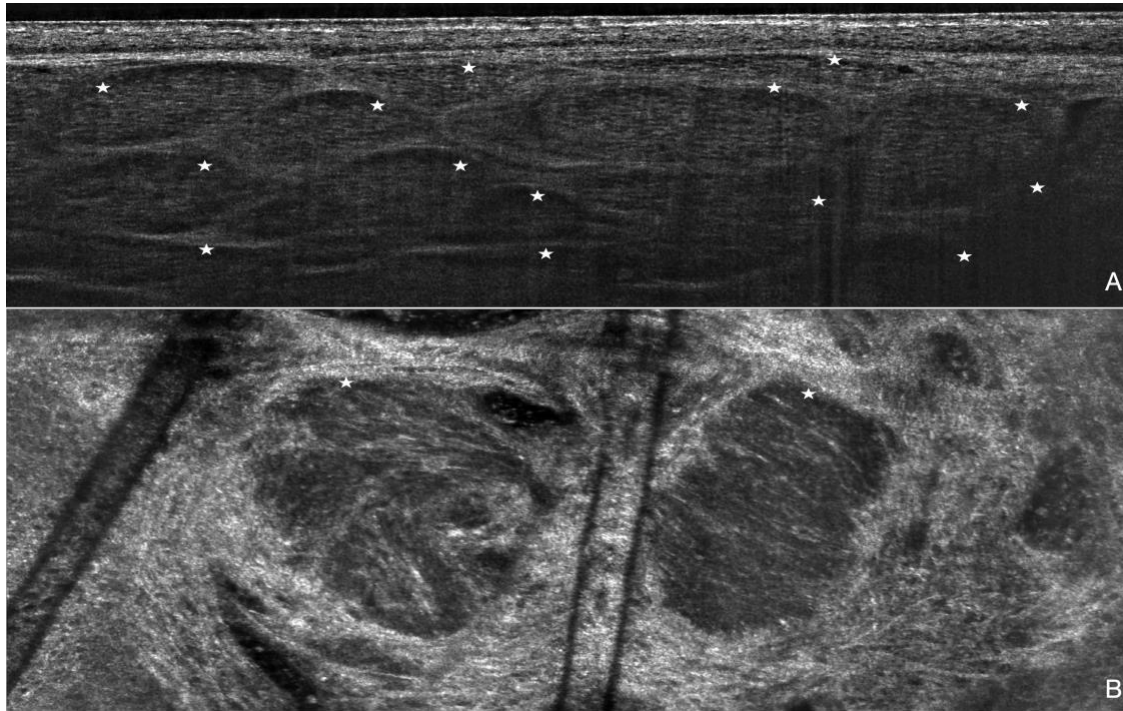


Figure 12. vertical (A) and horizontal (B) view of a BCC with the first LC-OCT prototype showing the typical basaloid tumor nests (star) with a peripheral clefting and collagen stromal reaction. Note the hyperreflective, fine, linear dendritic cells inside the nests.

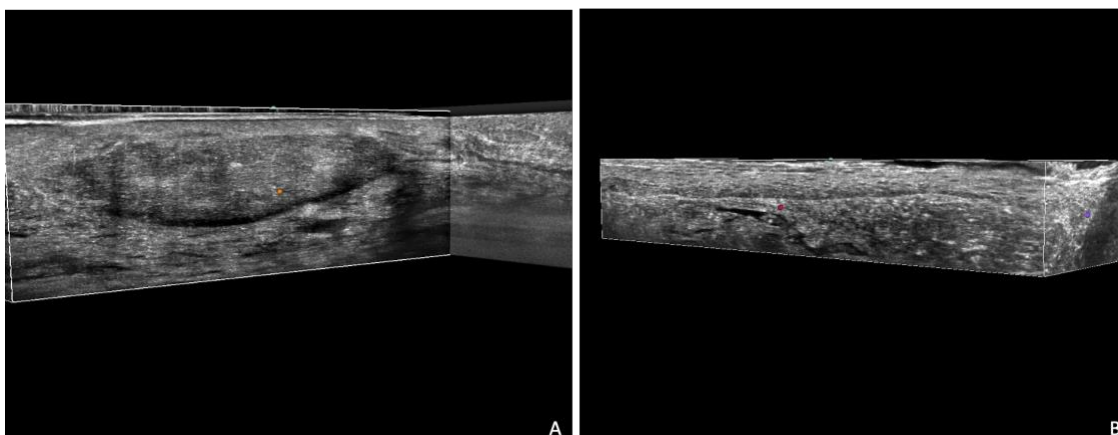


Figure 13. LC-OCT 3D reconstruction of a nodular-superficial (A) pigmented BCC and an infiltrative BCC (B) with ovoid hyporeflective structures (nests) with connection to the dermo-epidermal junctions (A) and infiltrating strands (B)

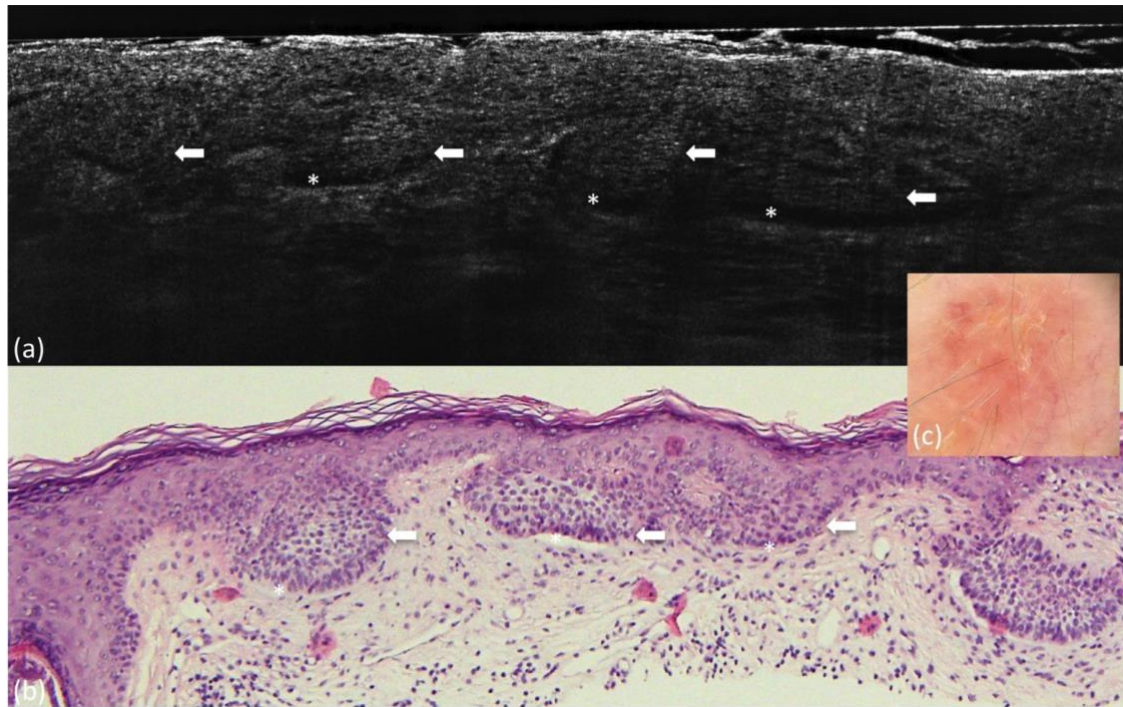


Figure 14. LC-OCT acquisition (A), H&E-stained histology 100x (B) and dermoscopy (C) of a superficial BCC. Note the hyporeflexive ovoid structures arranged in a string of pearls pattern (arrow), surrounded by dark rims corresponding to peripheral clefting (*). A hyperkeratotic crust (triangle) overlies the epidermal layer. Additionally, a sebaceous gland (rhombus) is visible.

(a) All lesions				
All lesions		LC-OCT		
		BCC	Non-BCC	Total
Histology	BCC	111	2	113
	Non-BCC	14	55	69
	Total	125	57	182
Dermoscopy				
		BCC	Non-BCC	Total
Histology	BCC	102	11	113
	Non-BCC	10	59	69
	Total	112	70	182

(b) High confidence lesions				
High confidence level for at least one of the 2 observers		LC-OCT		
		BCC	Non-BCC	Total
Histology	BCC	93	0	93
	Non-BCC	1	33	34
	Total	94	33	127
Dermoscopy				
		BCC	Non-BCC	Total
Histology	BCC	61	2	63
	Non-BCC	0	24	24
	Total	61	26	87

(c) Performance for BCC				
All BCC lesions				
N (%)	111 (100)			
Global accuracy	84%			
	Superficial BCC (N = 35)	Nodular BCC (N = 51)	Fibrosing BCC (N = 4)	Mixed (N = 21)
Accuracy	90%	88%	99%	90%
Sensitivity	77%	96%	75%	67%
Specificity	96%	82%	100%	96%
p value (Mac Nemar's test)	0.23	0.03	1	0.55
Good image quality				
N (%)	101 (91)			
Global accuracy	86%			
	Superficial BCC (N = 31)	Nodular BCC (N = 49)	Fibrosing BCC (N = 2)	Mixed (N = 19)
Accuracy	91%	90%	100%	91%
Sensitivity	81%	96%	100%	68%
Specificity	96%	85%	100%	96%
P value (Mac Nemar's test)	0.51	0.11	-	0.50
High confidence level for at least one of the 2 observers				
N (%)	93 (84)			
Global accuracy	84			
	Superficial BCC (N = 31)	Nodular BCC (N = 43)	Fibrosing BCC (N = 1)	Mixed (N = 18)
Accuracy	89%	89%	100%	89%
Sensitivity	77%	95%	100%	67%
Specificity	95%	84%	100%	95%
p value (Mac Nemar's test)	0.34	0.11	-	0.75

Table 4. LC-OCT diagnostic performance in all lesions (A), lesions with high diagnostic confidence (B) and for BCC (C)

Conclusions

LC-OCT enabled non-invasive real-time identification of BCCs and their subtypes in vertical, horizontal and 3D modes compared to histology, RCM and OCT. Our diagnostic confidence, sensitivity and specificity in detecting BCCs in the context of clinically equivocal lesions significantly improved using LC-OCT in comparison to dermoscopy only. Operator training for image acquisition is fundamental to achieve the best results.

Future perspectives are focused on the development of tools which are able to support clinicians for a quick, more objective and less operator-dependent bedside diagnosis. An ongoing multicenter study aims to train an artificial intelligence (AI) software to automatically recognize and color mark basaloid BCC nests and confounders during the live imaging procedure. (Figure 15)

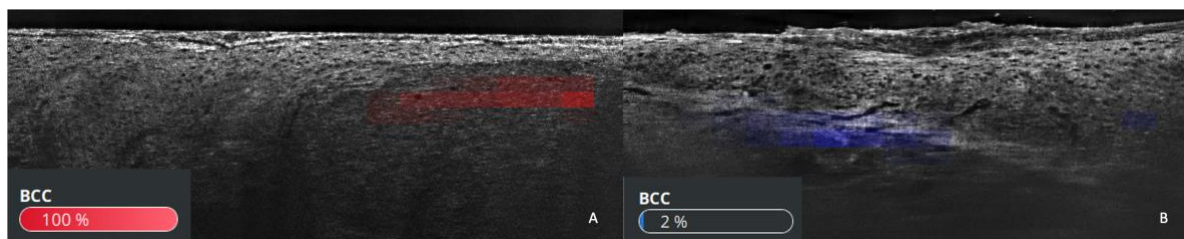


Figure 15. Nodular BCC (A) and AK (B); the built-in experimental software recognizes the typical ovoid nests of nodular BCC and color marks them in red while showing a high prediction score, whereas possible confounders are marked in blue in the heatmap.

IV. MELANOCYTIC TUMORS

Adapted from the original article: “Schuh S, Ruini C, et al. Line-Field Confocal Optical Coherence Tomography: A New Tool for the Differentiation between Nevi and Melanomas? Cancers (Basel). 2022 Feb 23;14(5):1140.”

Background

Although the typical benign nevus and advanced melanoma are easily distinguishable in the clinical practice, there are still some melanocytic lesions for which the correct diagnosis and grade are at first sight unknown, even for skilled specialists, so that non-invasive diagnostic tools were used to

overcome such hurdle. to this aim. RCM is known to have increased sensitivity and specificity of diagnosis of melanoma and dysplastic nevi over dermoscopy. It allows very good morphological discrimination of nevus or melanoma, but cannot indicate the depth of invasion of the lesion due to its low penetration depth. On the other hand, OCT can estimate lesion tumor thickness, but without cellular resolution, differentiation of melanocytic lesions remains impossible.

Aims

Since the novel LC-OCT theoretically bridges the gap between these RCM and OCT, this study explores whether LC-OCT can distinguish nevus from melanoma. The purpose was to evaluate whether LC-OCT is able to display typical morphological (cytological and architectural) features of melanocytic lesions compared to RMC and histology, and to determine whether it is possible to distinguish nevus from melanoma based on LC-OCT in-vivo examination.

Methods

A total of 84 melanocytic lesions were examined by LC-OCT and an additional 36 by RCM. Observers recorded the diagnosis and presence or absence of the 18 most common imaging parameters of melanocytic lesions, nevi and melanoma on LC-OCT images, based on commonly used histological and RCM criteria. Confidence in diagnosis and image quality of LC-OCT and RCM were assessed. We analyzed the sensitivity and specificity of the most useful criteria for distinguishing (dysplasia) nevi from melanoma, LC-OCT vs. RCM vs. histology.

Results

Better image quality correlated with better diagnostic performance (Spearman correlation: 0.4). (Figure 16) LC-OCT scored a 93% sensitivity and 100% specificity compared to RCM (93% sensitivity, 95% specificity) for diagnosing a melanoma compared to all types of nevi. Both devices misdiagnosed dysplastic nevi as non-dysplastic nevi (43% diagnostic sensitivity for dysplastic nevi). The most important criteria for the diagnosis of melanoma by LC-OCT were: irregular honeycomb

pattern (incidence 92%; 31.7 OR), presence of pagetoid spreading (incidence 89%; 23.6 OR), and dermal nests (23% incidence, 0.02 OR). (Table 5) (Figure 17, 18)

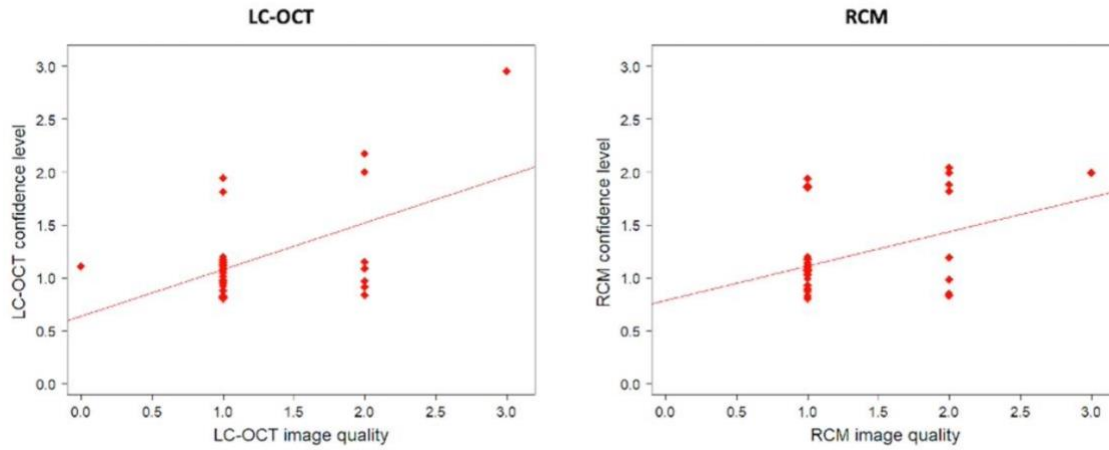


Figure 16. Positive correlation between diagnostic confidence and image quality for LC-OCT and RCM

LC-OCT Parameters	OR (Univariate)	<i>p</i> Value	OR (Multivariate)	<i>p</i> Value
Horizontal parameters				
Irregular honeycombed pattern	43.64 (10.80–299.67)	<0.001	18.03 (1.50–547.32)	0.039
Pagetoid spread with atypical melanocytes in basal/suprabasal layers	41.21 (11.28–206.45)	<0.001	16.56 (1.43–435.95)	0.037
Edged papillae	0.22 (0.08–0.59)	0.003	0.43 (0.02–9.05)	0.57
Basal nests	0.23 (0.07–0.63)	0.007	0.51 (0.02–12.06)	0.67
Nests in the upper dermis	0.21 (0.06–0.61)	0.007	0.23 (0.01–3.80)	0.35
Irregular bright cells/sheets of cells in the upper dermis	5.75 (1.77–20.94)	0.005	0.51 (0.02–10.73)	0.67
Vertical parameters				
Pagetoid spread of bright cells in suprabasal/basal layers	20.36 (6.44–74.84)	<0.001	4.74 (0.35–94.88)	0.25
Junctional nests	0.31 (0.11–0.83)	0.02	1.85 (0.06–103.11)	0.73
Disturbed DEJ	10.83 (3.73–35.72)	<0.001	4.85 (0.26–144.46)	0.31
Dermal nests	0.21 (0.07–0.58)	0.004	0.08 (0.00–1.29)	0.12
Sheets of atypical bright cells	26.47 (4.50–506.78)	0.003	0.69 (0.02–29.63)	0.83
RCM parameters				
Irregular honeycombed pattern	69.67 (9.13–1553.6)	<0.001	123.91 (2.84–19309)	0.030
Pagetoid spread with atypical melanocytes in suprabasal layers	34.00 (5.91–315.56)	<0.001	0.76 (0.01–23.85)	0.88
Edged papillae	0.08 (0.01–0.44)	0.007	0.05 (0.00–0.76)	0.053

Table 5. LC-OCT and RCM key criteria that were more useful in discriminating a melanoma from a nevus (dysplastic or not).

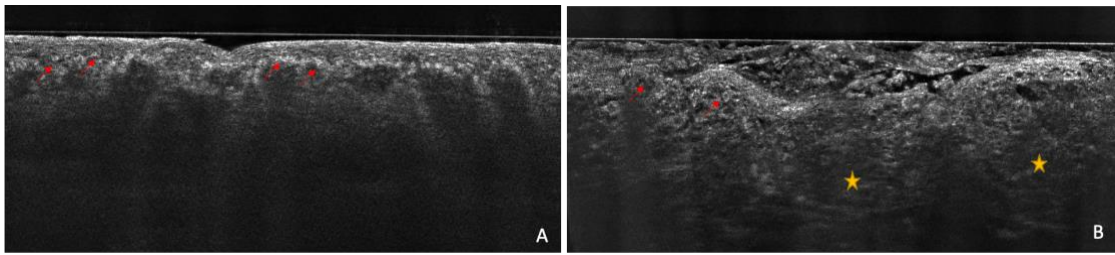


Figure 17. LC-OCT vertical acquisition of an in situ (A) and an invasive (B) melanoma; note the pagetoid spread (arrow) and the chaotic architecture with epidermal disruption and nests of atypical melanocytes (B)(*)

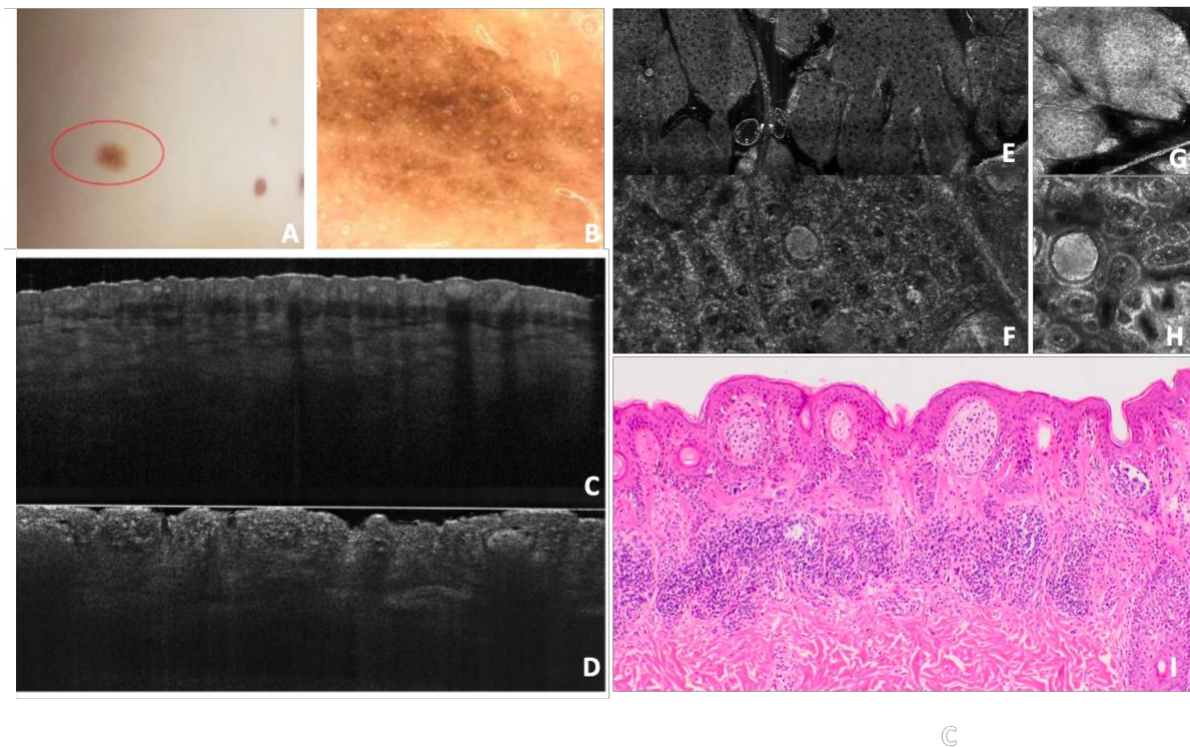


Figure 18. Compound nevus on the left lumbar trunk: Clinical (A), dermoscopic (B), OCT (C), LC-OCT acquisition in vertical mode (D) and horizontal mode at the epidermis (E) and DEJ (F), RCM acquisition at the epidermis (G) and DEJ (H), with corresponding H&E-stained histopathology (I) 4x. Note junctional and dermal nests, a well-defined DEJ, and a papillomatous surface can be seen in both OCT and LC-OCT, as well as a regular honeycomb pattern, with junctional nests, and regular papillae.

Conclusions

In summary, the first study with the new LC-OCT device on melanocyte lesions showed that it is potentially possible to distinguish between melanoma and nevus, potentially reducing unnecessary surgery and health insurance costs in the future. The visualization of horizontal and vertical sections provides additional and more intuitive details compared to RCM, however RCM still holds a higher

resolution which allows a more precise evaluation of melanocytic lesions when performed by experts. In this field, larger prospective studies are needed to better define diagnostic criteria and increase specificity and sensitivity as well as interobserver agreement.

V. PARASITIC INFESTATIONS

Adapted from the case reports “Ruini C, et al. In vivo imaging of *Sarcoptes scabiei* infestation using line-field confocal optical coherence tomography. *J Eur Acad Dermatol Venereol*. 2020 Dec;34(12):e808-e809” and “Ruini C, et al. Noninvasive real-time imaging of mite skin infestations with line-field confocal optical coherence tomography. *Br J Dermatol*. 2021 Jan;184(1):e3.

Non-invasive diagnostic tools are meanwhile well established in dermato-oncology, can however find potential applications in several other fields. Parasitic infestations are certainly one of them. Mites infestations are very common in dermatology; while *Demodex* mites are mainly saprophytes but play an important role in inflammatory diseases such as rosacea and demodex folliculitis, Scabies mites are responsible of endemic and epidemic scabies. Diagnosis is based on clinical features, dermoscopy, and scraping of the skin. In addition to RCM, a new imaging tool, confocal line-field optical coherence tomography (LC-OCT), provides real-time visualization of the skin in multiview images. *Demodex* bodies as visible as round hyperreflective structures inside hair follicles. (Figure 19) Scabies mites are clearly identifiable as ovoid structures with a thin corrugated exoskeleton and triangular spicules on the dorsal surface, located just below the SC within a cavity. Two pairs of hyperreflective cone structures corresponding to the legs surround the brighter head. A triangular terminal anus is visible on the caudal side. The internal organs are also shown with a central hyporeflexive tortuous gut with skybala and hyperreflective (probably ovarian and yolk) glands. Vertical and 3D sections allow the exact localization of the mite within the EP and SC; the burrow corresponds to a thick scale. Skybala and eggs are also recognizable as rounded, mostly hyperreflective structures. (Figure 20)

In addition to OCT and RCM, LC-OCT also allows fast, real-time, painless imaging of mites, their morphology, and their skin location; resolution is better than OCT, lower than RCM, pitfall that is mainly compensated by the possibility of vertical, horizontal and 3D reconstructions.

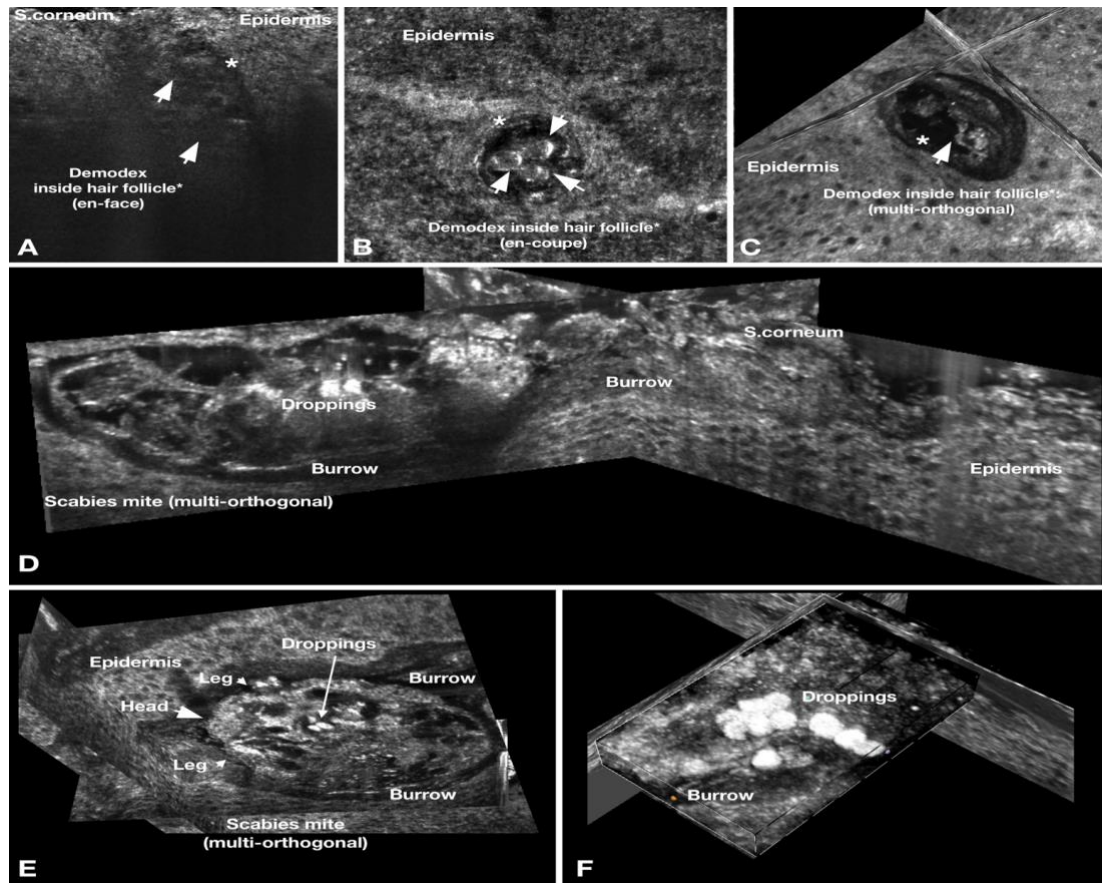


Figure 19. Demodex (a–c) and scabies mites (d–f) in multiview. Demodex mites appear as round hyper-reflective structures inside the hair follicles (a–c), while scabies mite as ovoid structures inside the dark burrows with hyper-reflective droppings (d–f)

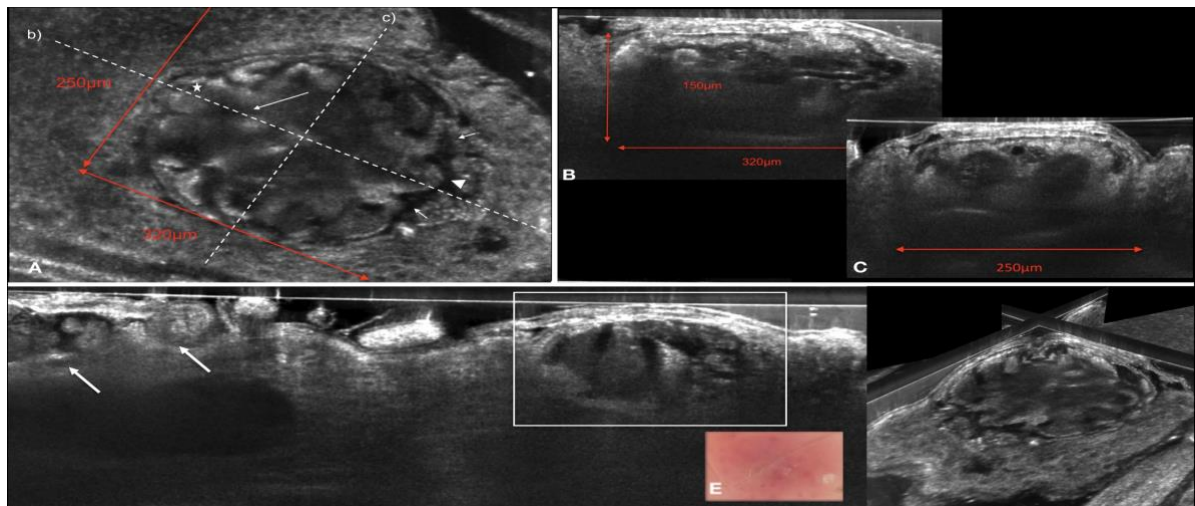


Figure 20. a) In en-face LC-OCT images, *S. scabiei* can be identified as a round structure in a burrow with a thin, corrugated exoskeleton and triangular spicules on the dorsal surface. Two pairs of hyperreflective coned structures (short arrows) corresponding to legs surround the polygonal head (triangle). At the opposite side, a triangular terminal anus (star) is visible and in the centre, a central, hyporeflective, convoluted gut (long arrow) with skybala and hyperreflective glands can be seen. 1b,c) Vertical LC-OCT images corresponding to the b) and c) axes in the 1A en-face image 1d) Vertical LC-OCT image with a thick hyperkeratotic burrow surrounding the mite inside the epidermis. The ovoid burrow behind the mite contains the hyperreflective skybala and eggs. 1e) Dermoscopy of the scabies mite with the so-called 'delta' or 'kite' or 'triangle' sign 1f) 3D reconstruction of the hyperreflective burrow containing the mite body.

V. INFLAMMATORY SKIN DISEASES: FIRST IMPRESSIONS

Unpublished data

Introduction

Line-field confocal optical coherence tomography (LC-OCT) is a novel non-invasive diagnostic tool that has recently been used in the bedside diagnosing and grading of non-melanoma skin cancer. It combines cellular resolution and penetration depth up to the mid-dermis in both vertical and horizontal sections. Its use on inflammatory skin diseases is yet to be explored systematically.

Objectives

To assess preliminary LC-OCT in-vivo correlates of the main histological patterns of inflammatory skin diseases (eczematous, lichenoid, bullous, psoriatic, fibrotic and granulomatous)

Material and Methods

One hundred inflammatory skin lesions were enrolled in the study. Each lesion was imaged using dermoscopy, OCT, LC-OCT and confocal microscopy. The LC-OCT device is based on a two-beam interference microscope with line illumination allowing three imaging modalities: vertical, en-face and 3D. Acquired diagnosis were: bullous pemphigoid, pemphigus vulgaris, lichen ruber, psoriasis vulgaris, psoriasis pustulosa, acute contact dermatitis, atopic dermatitis, chronic discoid lupus erythematosus.

Results

The preliminary results of this study focused on the identification of in-vivo morphological patterns typical for inflammatory and bullous skin diseases. LC-OCT could visualize with the combination of horizontal and vertical sections imaging patterns corresponding to typical histological features such as spongiosis, blistering and inflammatory infiltrate (eczema) (Figure 21), hyperkeratosis, elongated rete ridges, glomerular dilated vessels (psoriasis) (Figure 22), intraepidermal blistering (pemphigus vulgaris), subepidermal blistering (bullous pemphigoid). (Figure 23)

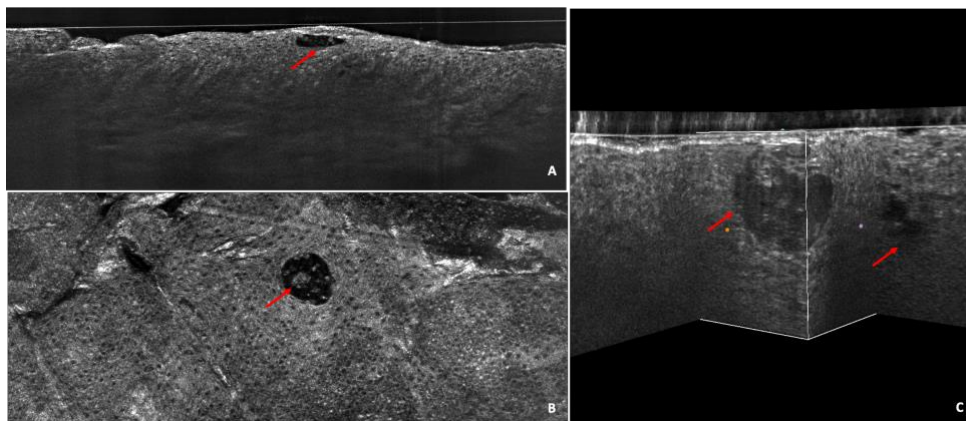


Figure 21. LC-OCT image of an acute contact dermatitis (positive patch test reaction to nickel) in vertical (A), horizontal (B) and 3D (C) mode: note dilated intracellular spaces (spongiosis), intraepidermal hyporefective cavities filled with hyperreflective floating elements (blistering and inflammatory cells) (arrows)

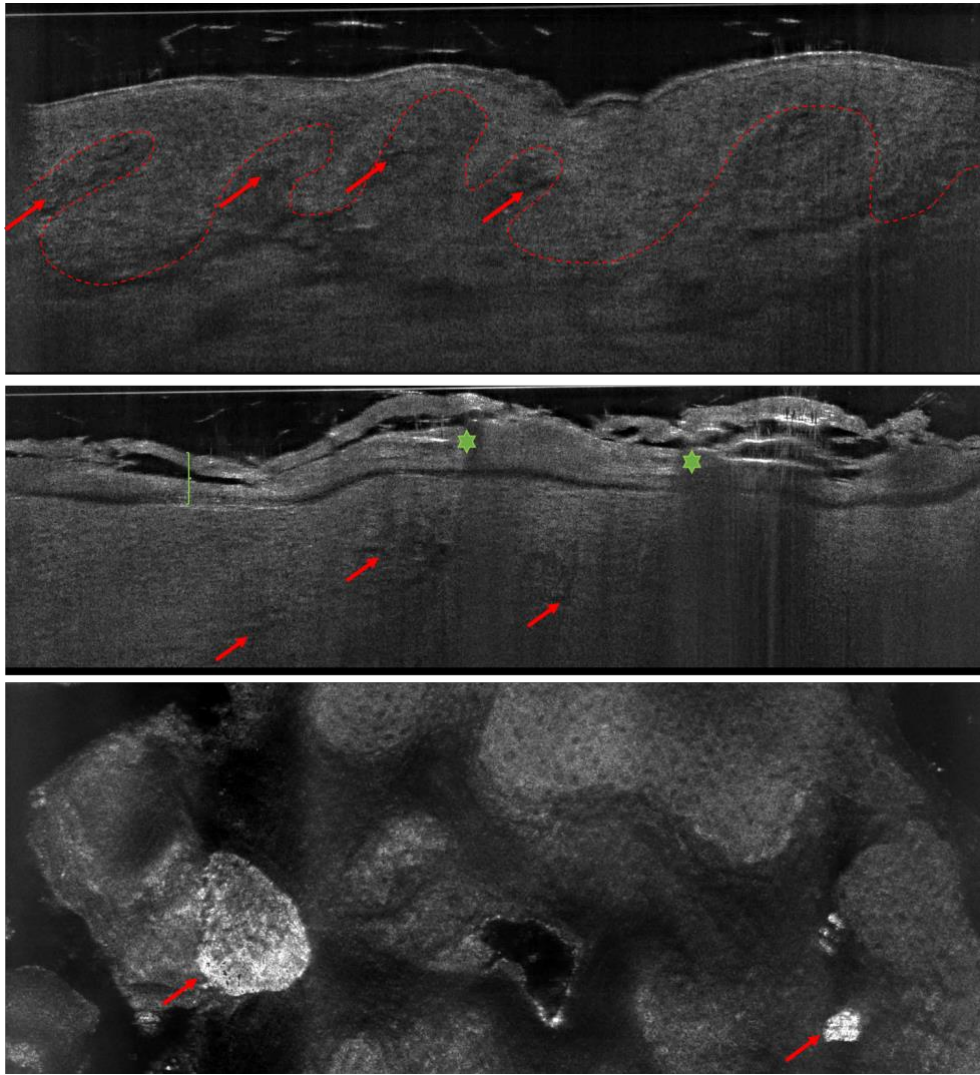


Figure 22. LC-OCT acquisition of psoriasis vulgaris lesions in vertical (A,B) and horizontal (C) mode: note the elongated rete ridges (dotted line) with dilated capillaries inside the papillae (arrows), hyperkeratosis (*), acanthosis with hyporeflective roundish structures (Munro's microabscesses)(arrows)

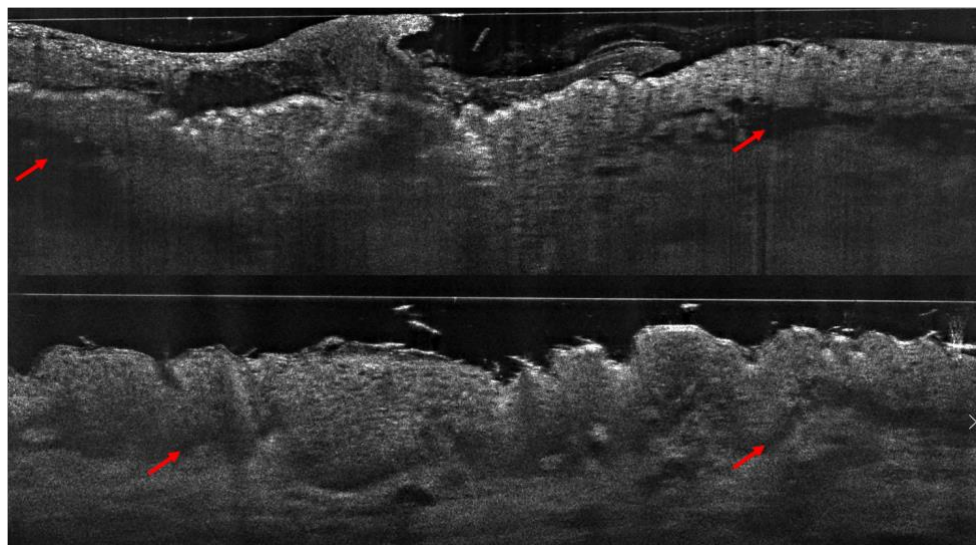


Figure 23. LC-OCT image of bullous pemphigoid lesions in vertical mode (A,B): a crust (*), subepidermal blistering (arrow).

Conclusions

LC-OCT is able to display selected histopathological correlates of inflammatory skin diseases, and can potentially integrate diagnosis and follow up. Moreover, it is very fast, painless, offers real time measurements during the patient visit and also enough penetration depth like OCT for the visualisation of the junctional and dermal layers. Large prospective studies integrating LC-OCT, conventional OCT and confocal microscopy as well as artificial intelligence are necessary to better investigate this yet unexplored field of application of non-invasive diagnostic techniques.

CONCLUSIONS

The constant development of modern technologies, the interest in reducing unnecessary invasive procedures together with the increasing health awareness of patients have led to an extensive use of non-invasive diagnostic techniques, especially in the early diagnosis of skin tumors, and the search for new areas of application of these outside dermato-oncology. In this work, we explored the applications of the novel device LC-OCT following its evolution prototype after prototype, and compared its advantages and pitfalls to well established diagnostic techniques (dermoscopy, OCT and RCM). This work contributes to a better understanding of the above methods and their use. Furthermore, it deals with aspects directly relevant to practice and therapy in the everyday care of patients with skin cancers. In the daily clinical practice, diagnosis of skin tumors is usually done by clinical examination and dermoscopy, followed by surgical biopsies to confirm clinical suspicion. Biopsies are expensive procedures that does not always lead to the correct diagnosis; a large or multilocular lesion, for example, may be underestimated or misdiagnosed by minimal tissue sampling. A hot topic of contemporary dermatology is the improvement of early diagnosis in order to minimize invasive surgical treatments, and to reduce the significant socio-economic damage. It is now known, for example, that early detection and treatment of skin cancers provides increased

survival, improved quality of life and reduced treatment costs. Even in the case of non-oncological skin diseases, unnecessary surgical biopsies should be avoided, but clinical diagnosis is sometimes complex, especially in the early stages.

On these grounds, the aim of this work was to improve dermatological diagnostics in order to establish faster, safer and more beneficial diagnostic procedures, as well as to explore new areas of application for them.

In this work we were able to investigate the innovative and so far hardly explored technique LC-OCT, to determine the non-invasive diagnostic criteria and correlate them to the already established RCM, OCT and H&E sections. Images of healthy skin and various skin tumors were compared with the respective conventional histological slides to elaborate the features of the investigated entities; thus, a correct diagnosis of skin lesions can be made non-invasively in the future. The main limitations were also critically analyzed. We were able to confirm a high accuracy for the diagnosis and subtyping of BCC and field cancerization, as well as an improvement in diagnostic confidence compared to other diagnostic techniques. This can lead to significant advantages in clinical practice, as the correct classification allows unnecessary biopsies to be avoided and patients to be referred directly for the correct treatment (non-operative for superficial tumors, operative for nodular and fibrosing entities). LC-OCT demonstrated to be not inferior to other well established diagnostic techniques such as dermoscopy, RCM and OCT, at least in NMSC. We additionally proved that LC-OCT can classify AK based on the basal growth pattern of keratinocytes, reproducing the histological PRO classification in vivo. Since this correlates with the risk of progression to SCC, it can be assumed that the malignant potential of AK could be estimated non-invasively in the future.

There exist of course several limitations. LC-OCT is very intuitive compared to RCM, for example, but requires detailed knowledge of the histology and pathology of the skin to interpret the images correctly, so this technique should also definitely be learned in the form of training courses.

Resolution is nearly cellular, although single cells are sometimes difficult to assign nosologically, especially inflammatory cells. The depth of penetration to the mid dermis causes lack of assessment of deeper dermal changes, especially in thicker lesions, such as nodular melanoma, nodular basal cell carcinoma or panniculitis. The methodology remains user-dependent, and the area examined is difficult to standardize, especially for follow-up imaging. Three-dimensional reconstruction is currently not integrated in the imaging mode and requires time-consuming elaboration with appropriate software.

Nevertheless, the innovative imaging procedures in dermatology have made great progress in recent years. They could not only be used for the early detection and classification of skin cancer, but also for the correct diagnosis of numerous skin diseases and the improvement of bedside histology. However, they still harbor many limitations and pitfalls, especially concerning low penetration depth and low resolution, as well as operator dependent handling. Far from wanting to replace the experienced clinicians, these new diagnostic techniques are intended to support them in their daily routine.

As non-invasive diagnostics is an innovative and promising field of research, we expect the further development of already existing techniques as well as the establishment of new methods that can improve our clinical eye. Based on this, the following goals arise for future work, for example: the systematic review of LC-OCT criteria for skin tumors and inflammatory dermatoses in multicenter studies, the use of artificial intelligence in in-vivo and ex-vivo diagnostics.

Our publications represent fundamental work in the field of LC-OCT, which will most likely lead the way for future studies on this topic. It is expected that the increasing optimization of this technique and its dissemination, together with the steadily growing interest of patients and clinicians, will lead in the long term to a significant improvement in early diagnosis as well as therapy monitoring of skin tumors and numerous dermatoses.

LIST OF ABBREVIATIONS

AC	Attenuation coefficient
AK	Actinic keratosis
BCC	Basal cell carcinoma
BD	Bowen Disease
CI	Confidence interval
DEJ	Dermo-epidermal junction
EP	Epidermis
fBCC	Fibrosing BCC
H&E	Hematoxylin-Eosin
KC	Keratinocyte cancers
LC-OCT	Line-field confocal optical coherence tomography
LM	Lentigo maligna
LMM	Lentigo maligna melanoma
MM	Malignant melanoma
nBCC	nodular BCC
OCT	Optical coherence tomography
PDT	Photodynamic therapy
RSOM	Raster-Scanning Optoacoustic Mesoscopy
sBCC	superficial BCC
SC	Stratum corneum
SCC	Squamous cell carcinoma
SG	Stratum granulosum
SS	Stratum spinosum

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SELECTION OF PUBLICATIONS DERIVED FROM THIS PROJECT

Line-field confocal optical coherence tomography—Practical applications in dermatology and comparison with established imaging methods

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Abstract

Background: Non-invasive diagnostic techniques in dermatology gained increasing popularity in the last decade. Reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) are meanwhile established in research and clinical routine. While OCT is mainly indicated for detecting non-melanoma skin cancer, RCM has proven its usefulness additionally in distinguishing melanocytic lesions. Line-field confocal optical coherence tomography (LC-OCT) is an emerging tool combining the principles of both above-mentioned methods.

Methods: Healthy skin at different body sites and exemplary skin lesions (basal cell carcinoma, malignant melanoma, actinic keratosis) were examined using dermoscopy, RCM, OCT and LC-OCT. Standard features for RCM and OCT and comparable features for LC-OCT were analysed.

Results: LC-OCT has a lower penetration depth but superior resolution compared to OCT. In comparison with RCM, which provides only horizontal sections, LC-OCT creates both vertical and horizontal images in real time and has nearly the same cellular resolution.

Discussion: Our preliminary experiences suggest that LC-OCT combines the advantages of RCM and OCT, with optimal resolution and penetration depth to diagnose all types of skin cancer.

Larger systematic studies are needed to further characterize the field of use of this device and its sensitivity and specificity compared to histology.

KEYWORDS

dermatology, line-field confocal OCT, optical coherence tomography, reflectance confocal microscopy, skin cancer, skin imaging

Cristel Ruini, Sandra Schuh, Elke Sattler and Julia Welzel contributed equally.

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1 | INTRODUCTION

Optical coherence tomography (OCT) is well established in non-invasive diagnostics, especially for epithelial skin tumours. It enables the visualization of architectural changes down to the middle dermis, but without cellular resolution. Primarily, depth section images are displayed, whereby three-dimensional overview images are possible. This allows basal cell carcinomas (BCCs) to be diagnosed and distinguished from other epithelial tumours and precancerous lesions. It is also possible to determine the tumour thickness and the subtype of basal cell carcinoma. In the case of non-surgical therapies such as photodynamic therapy or topical imiquimod, OCT is also suitable for monitoring the course of the disease.¹⁻⁵

Reflectance confocal microscopy (RCM) has also been established in routine diagnostics for many years. Its domain is the differential diagnosis of pigmented lesions, as it offers a high resolution with visibility of cytological details. However, its penetration depth is limited to the stratum papillare of the dermis, missing deeper parts of tumours. RCM provides horizontal images of small sections that can be combined to form a larger mosaic.^{6,7}

Line-field confocal optical coherence tomography (LC-OCT) allows the simultaneous display of horizontal and vertical images with a higher resolution than conventional OCT, but also with a higher detection depth compared to RCM.

A LC-OCT prototype (DAMAE, Paris) was used in this study to image healthy skin at different locations, to display a basal cell carcinoma, an actinic keratosis and a malignant melanoma exemplarily and to compare the findings with OCT and RCM. The intention was to evaluate the potential of LC-OCT in comparison to OCT and RCM regarding diagnosis of skin cancer.^{8,9}

2 | MATERIALS AND METHODS

2.1 | Imaging devices

2.1.1 | LC-OCT

The LC-OCT system is based on a two-beam interference microscope with line illumination and line detection using a central wavelength of spatially coherent light source and a line-scan camera. The light source is classified as a class 1 supercontinuum laser with a central wavelength of 800 nm. The real-time image acquisition is non-invasive, painless for the patient and provides no tissue damage, so that it is also allowed to be applied in pregnant women and children.

LC-OCT measures the time of flight and amplitude of light, which is backscattered from the tissue microstructures. It substantially combines the principle of OCT interferometry with the spatial filtering of RCM. The device collects multiple A-scans in parallel from the skin surface to a depth of ~500 µm, constantly adjusting the focus. The LC-OCT device (DAMAE Medical, Paris)

has three imaging modalities: vertical or en-coupe (similar to OCT and histology), en-face (comparable with RCM) and 3D stack for a three-dimensional reconstruction. Both short movies and stacks from surface to depth can be recorded. It has an axial resolution of 1.1 µm, a lateral resolution of 1.3 µm and a field of view of 1.2 mm × 0.5 mm (vertical) and 1.2 mm × 0.5 mm (en-face), respectively. Images are displayed in a grey scale. It provides a simultaneous live acquisition and imaging evaluation, thanks to its acquisition speed of 10 images/second (2D) resp. 15 seconds for a 3D stack. The device consists of a central unit connected to a handheld probe and a monitor, with Bluetooth keyboard and mouse. Immersion oil is applied between the glass window of the probe and the skin surface for index matching. It is provided with a wheeled cart, so that it can be easily moved towards the patient. Complete technical details are described elsewhere.¹⁰⁻¹²

2.1.2 | RCM

The commercially available RCM (VivaScope® 1500 and 3000, Mavig GmbH, Munich, Germany), is based on a near-infrared, low-power laser beam (830 nm diode laser, up to 35 mW). The lateral resolution is 1 µm, the axial resolution is approximately 3-5 µm. The penetration depth reaches the superficial dermis at 200-250 µm. Skin images are displayed in a grey scale, where melanin and keratin serve as endogenous chromophores, provide the most contrast and appear bright, in contrast to water (cytoplasm), which appears black. There are essentially two available types of the device (VivaScope® 1500, fixed to a flexible arm, and VivaScope® 3000, a handheld probe) and a combination of both of them. The 1500 device has a field of view of 500 × 500 µm and can build a mosaic grid of single greyscale contiguous images up to 8 × 8 mm (VivaBlock®); a series of vertical stacks from the surface to the depth (VivaStack®) can also be acquired. VivaScope 1500 offers an integrated dermoscopic camera to allow navigation in the mosaic and immediate comparison with dermoscopy. The newest generation has a built-in camera for both clinical and dermoscopic pictures.

In our study, images sized 500 × 500 µm up to 200 µm in depth were collected using the VivaStack® function with a commercially available reflectance confocal microscope (VivaScope® 1500 and 3000, Mavig GmbH, Munich, Germany).

Further details on the device are described elsewhere.¹³

2.1.3 | OCT

Three-dimensional OCT images sized 6 × 6 mm down to a skin depth of about 1.5 mm were acquired using the commercially distributed Vivosight® (Michelson Diagnostics Ltd, Maidstone, Kent, UK). This frequency domain OCT is based on Michelson interferometry, has a lateral resolution of 7.5 µm and an axial resolution

of 10 μm . Its laser source (HSL 2000; Santec Corporation, Komaki, Japan) has a central wavelength of 1,305 nm. The device has a handheld probe, which is provided with a series of plastic spacers to adjust the focus to different skin sites. The dynamic mode allows the visualization of blood flow simultaneously with the morphologic features of the tissue.

A more detailed description of the device is provided elsewhere.¹

2.1.4 | DERMOSCOPY

Clinical and dermoscopic images of each lesion were acquired using FotoFinder (FotoFinder Systems GmbH, Bad Birnbach, Germany) and a DermoGenius 2 (DermoScan GmbH, Regensburg, Germany) as well as an ILLUCO IDS-1100 (DermoScan GmbH, Regensburg, Germany).

2.2 | Subjects

The study was approved by the local ethics committee (Nr17-699), and informed consent was obtained from each subject. The study was conducted according to the principles of the declaration of Helsinki and international guidelines concerning human studies. Two women aged 33 and 49 were enrolled in the study for measurements of healthy skin in three different locations. In addition, one woman aged 51 with an actinic keratosis on the nose, one man aged 89 with a nodular BCC on his right eyebrow and one man aged 78 with a malignant melanoma on his right thigh were examined in the study.

2.3 | Measurements

Healthy skin at different locations (right cheek, right volar forearm and right middle fingertip as well as exemplary neoplastic skin diseases) were investigated. The following images were collected for each site: LC-OCT (en-face, vertical and 3D), OCT (vertical and dynamic en-face), RCM (VivaBlock[®] and VivaStack fully described elsewhere¹⁴). An adhesive ring marked the measuring position and guaranteed that exactly the same spot was measured with each method. Skin lesions except for healthy skin were compared with their standard histology in haematoxylin/eosin. Only good quality images were included into the study. Subjects were sitting or lying comfortably while the operator performed the measurements. On the test site, the skin was pre-treated with immersion oil to reduce light reflection from the surface for LC-OCT and RCM, whereas the OCT images were collected without pre-treatment.

Following skin layers were individually evaluated: stratum corneum (SC), epidermis (EPI), dermo-epidermal junction (DEJ), papillary dermis (PD) for all three methods, and reticular dermis (RD) only in OCT.

The measurements were performed between November 2019 and March 2020 at the dermatology departments of the university hospitals of Munich and Augsburg.

3 | RESULTS

3.1 | Healthy skin

The correlation between LC-OCT images and haematoxylin-eosin sections of normal skin can be seen in Figure 1A vs. B. In

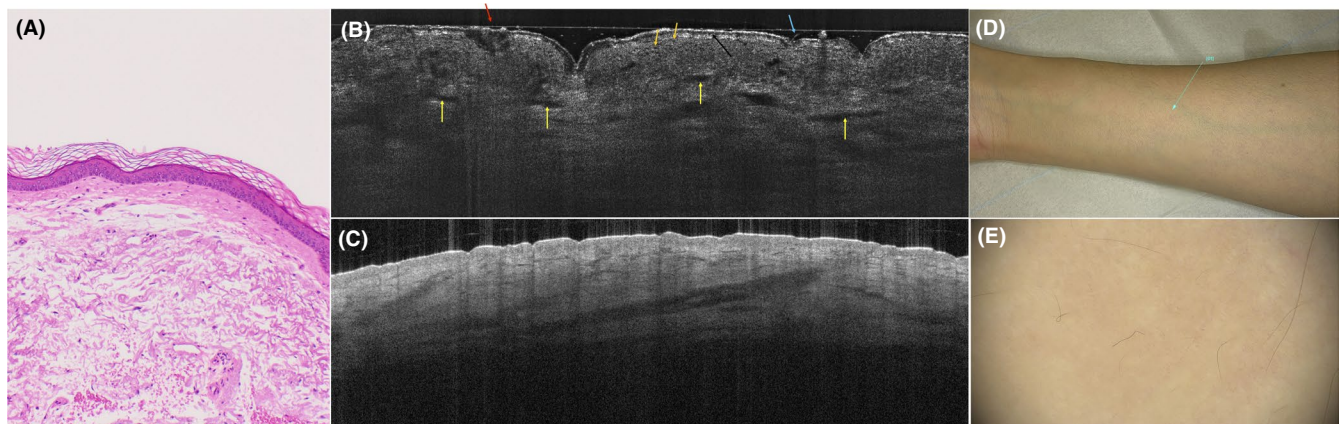


FIGURE 1 Comparison of HE sections, vertical LC-OCT images and OCT images of a healthy right forearm. (A) Haematoxylin-eosin section of normal skin on the right forearm (10 $\times$ magnification). (B) LC-OCT image (1.2 $\times$ 0.5 mm) of healthy skin on the right forearm shows that the SC is composed of corneocytes, and detached hyperreflective scales are visible (blue arrow), too. Erosions appear as a dark and irregular discontinuity of the surface (red arrow). Keratinocytes are slightly darker polygonal cells, showing bright contours (orange arrows) and decrease from SC to stratum spinosum (orange area). Hair follicles are visible as dark longitudinal shadows (green area), sometimes with a bright hair shaft (green arrow). The DEJ is a thin dark line constituted of a layer of small basal cells (white arrows), which separates the epidermis from the hyperreflective papillary dermis. Blood vessels are represented as hyporefective linear and convolute structures (yellow arrows). (C) The same healthy skin on the right forearm (6 $\times$ 1 mm) in OCT, which shows less architectural and cytological details (D) shows the macroscopical area on the forearm and (E) its dermoscopic correlate [Colour figure can be viewed at wileyonlinelibrary.com]

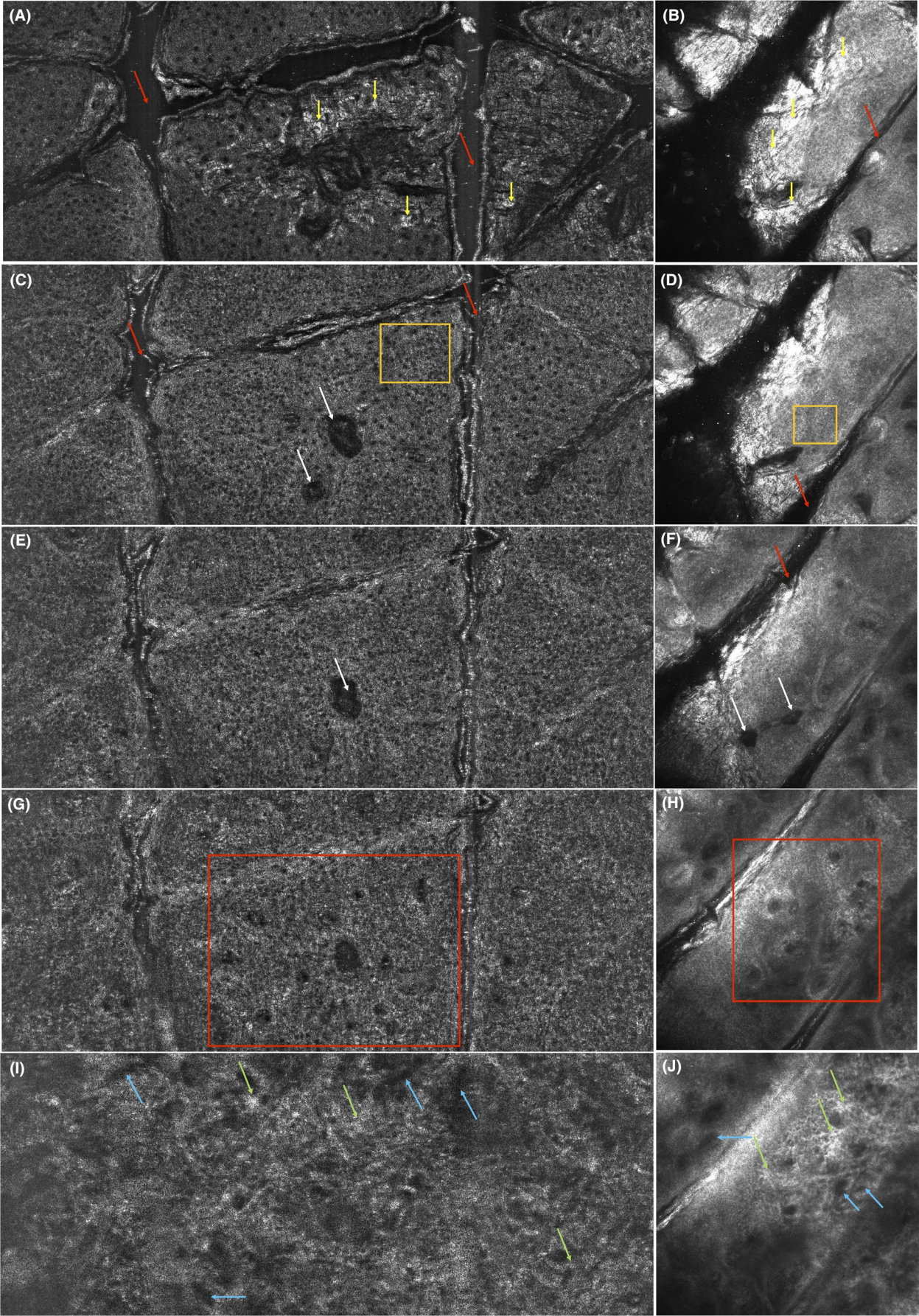


FIGURE 2 Comparison of RCM and en-face mode LC-OCT images of a healthy right forearm. (A) In en-face mode (0.5×1.2 mm) of the LC-OCT images, hyperreflective single corneocytes of $10\text{--}30\text{ }\mu\text{m}$ can be seen in the SC (yellow arrows) like in RCM (B). (C) The keratinocytes composing the EPI are polygonal cells with dark nuclei and appear in a regular honeycombed pattern (orange area). The same can be seen in RCM images (D). Skin folds are sharply demarcated, non-refractile linear furrows between groups of keratinocytes (red arrows) in (A and C) but also in RCM images (B, D, F). In (C and E) in LC-OCT images as well as in (F) in RCM, hair follicles are also very well distinguishable concentric round structures, with a dark, round, central opening surrounded by a multilayer of darker epithelial cells (white arrows). (G and H): The DEJ in LC-OCT and RCM images of normal skin has a regular ringed architecture of dark round to oval dermal papillae centred by dermal capillary loops, (red area). (I and J) In the PD in LC-OCT, but also in RCM hyperreflective collagen fibres and bundles (green arrows) and hyporeflexive linear and roundish blood vessels dominate (blue arrows) [Colour figure can be viewed at wileyonlinelibrary.com]

LC-OCT, single cells and even nuclei can be distinguished as well as follicular openings and blood vessels. En-face and vertical images showed significant variations depending on the anatomical

location. LC-OCT features in vertical mode were visible in OCT, too, although LC-OCT allows a much better definition of cytological details (Figure 1B vs. C).

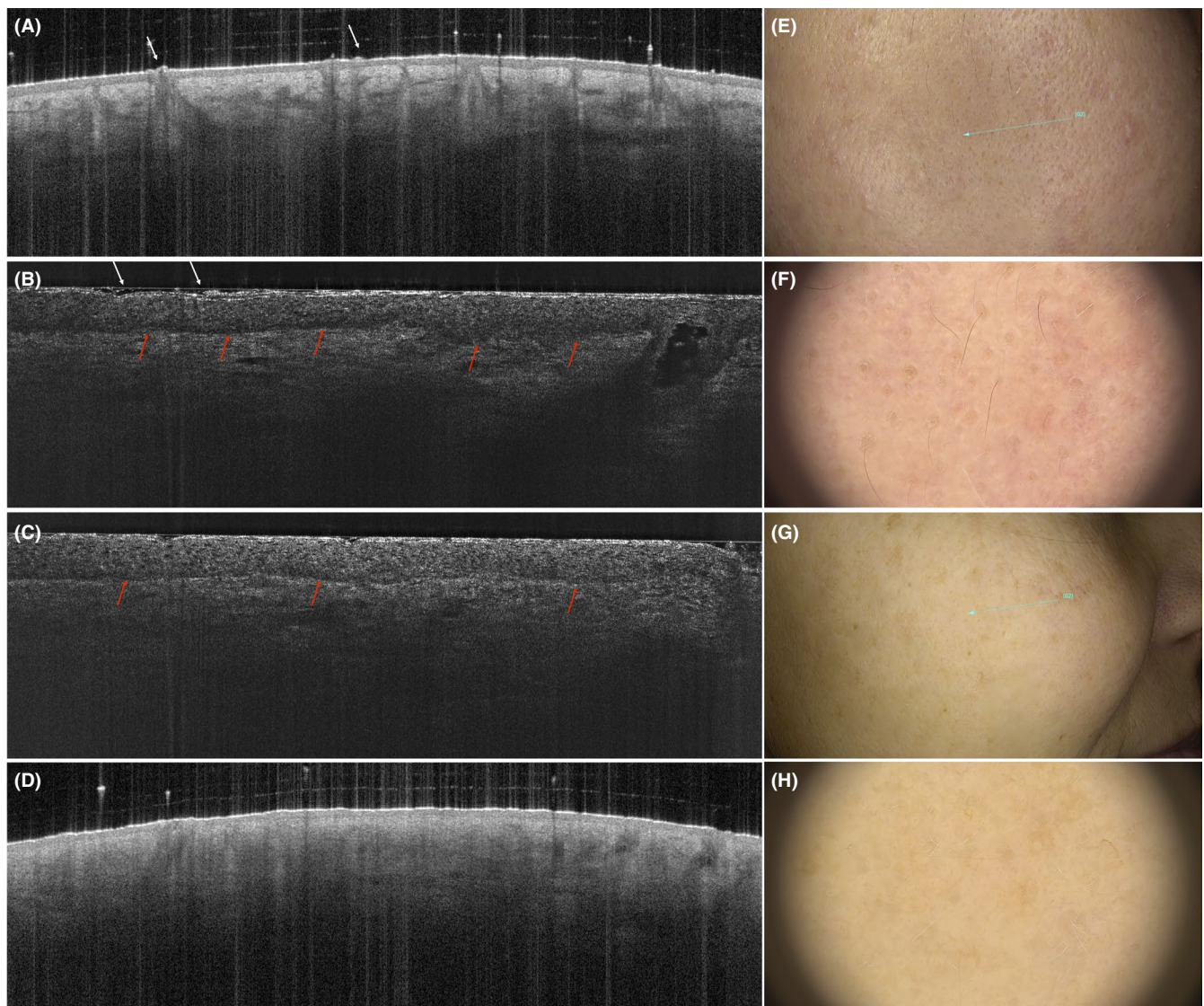


FIGURE 3 Comparison of vertical LC-OCT images and OCT images of the right cheek of a younger (clinical and dermoscopic correlates are shown in E and F, respectively) and an elderly woman (clinical and dermoscopic correlates are shown in G and H, respectively). (A, B) The surface of the (thin) SC, EPI and DEJ in vertical mode appears to be slightly undulated (white arrows) in OCT and LC-OCT. Sebaceous glands appear as globular bright structures connected to the epidermis, sometimes with an opening into hair follicles (yellow area in B). Demodex mites are visible inside the hair follicles as hyperreflective globules in vertical mode (yellow arrow in B). (C, D) In elderly skin, the DEJ is slightly more flattened (red arrows). The dermis is brighter in younger subjects compared to older ones, probably due to the more compact structure of collagen fibres (B vs. C). The latter appears more rarefied and therefore darker in elderly skin (C) [Colour figure can be viewed at wileyonlinelibrary.com]

Figure 1B shows that the SC in vertical mode is composed of corneocytes; sometimes detached hyperreflective scales are visible. Erosions appear as a dark and irregular discontinuity of the surface. Keratinocytes are slightly darker polygonal cells, showing bright contours. They decrease in size passing from SC to stratum granulosum to stratum spinosum. Hair follicles are visible as dark longitudinal shadows, sometimes with a bright hair shaft. The DEJ is a thin dark line constituted of a layer of small basal cells. It separates the clearly distinguishable epidermis from the papillary dermis, the latter appearing hyperreflective. Blood vessels are represented as hyporeflective linear and convolute structures.

In the en-face mode in Figure 2A, hyperreflective single corneocytes of 10–30 μm are displayed on the surface in the SC like in RCM in Figure 2B. The keratinocytes composing the EPI are polygonal cells with dark nuclei and are arranged in a sort of honeycombed pattern appearing regular in Figure 2C and D. More pigmented keratinocytes appear brighter and create a cobblestone pattern. Skin folds are sharply demarcated, non-refractile linear furrows between groups of keratinocytes and can be seen in Figure 2A and C in LC-OCT as well as in Figure 2B, D, F in RCM. Hair follicles are also very well distinguishable concentric round structures, with a dark, round, central opening surrounded by a multilayer of darker epithelial cells like in 2C and E in LC-OCT and in 2F in RCM images. The DEJ of normal skin has a regular ringed architecture, composed of dark round to oval dermal papillae centred by capillary loops, depending on the transversal section of the dermal papillae. These correspond to the edged papillae visible in RCM at the

DEJ (in 2 h in RCM images). In 2I in LC-OCT images as well as in 2J in RCM images, hyperreflective collagen fibres and bundles and hyporeflective linear and roundish blood vessels dominate in the PD.

The LC-OCT en-face and vertical images showed significant differences depending on the location, as expected. The above-mentioned main morphological patterns of healthy skin in LC-OCT seem to correspond to the respective OCT and especially to the RCM features.

3.1.1 | Cheek

As common on adnex-rich skin, the surface of the (thin) SC, EPI and DEJ in vertical mode appears to be slightly undulated (white arrows) in Figure 3A in OCT and in B in LC-OCT. Sebaceous glands appear as globular bright structures connected to the epidermis, sometimes with an opening into hair follicles (yellow area in Figure 3B). Some bright, roundish and homogeneous comedones, filled with amorphous material, can also be seen (yellow arrows in 4 d). Demodex mites as skin commensals are visible inside the hair follicles as hyperreflective globules in vertical mode and as large, grouped, hyperreflective structures in en-face mode (yellow arrow in Figure 3B, white arrows in Figure 4A and E). The DEJ is slightly more flattened in elderly skin (red arrows) in Figure 3C in LC-OCT and in Figure 3D in OCT. The dermis is brighter in the younger subject compared to the older one, probably due to the more compact structure of collagen fibres (Figure 3B vs. C). The

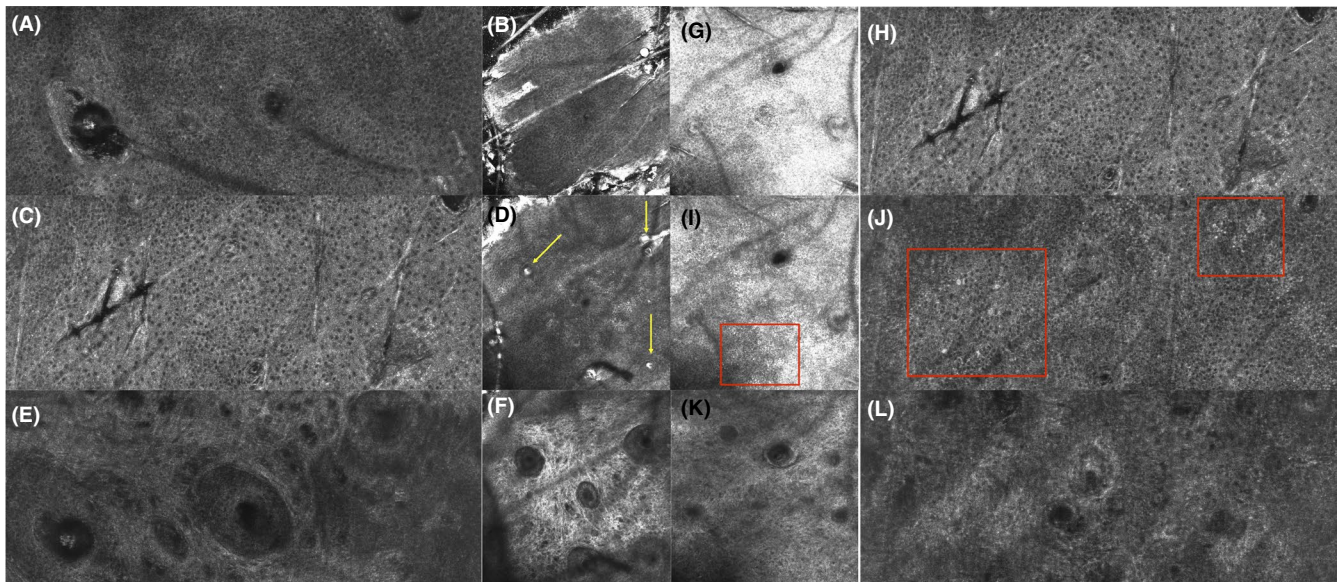


FIGURE 4 Comparison of en-face LC-OCT images and RCM images of the right cheek of a younger and an elderly woman. Some bright, roundish and homogeneous comedones, filled with amorphous material, can also be seen (yellow arrows in D). Demodex mites as skin commensals are visible inside the hair follicles as hyperreflective globules in vertical mode and as large, grouped, hyperreflective structures in en-face mode (yellow arrow in Figure 3B, white arrows in A Figure 4A and E). The dermis appears more rarefied and therefore darker in elderly skin (Figure 4K in RCM and Figure 4L in LC-OCT) compared to the younger woman (Figure 4E in LC-OCT and Figure 4F in RCM). Signs of sun damage, more represented in elderly skin, like mottled pigmentation (red areas in Figure 4J in LC-OCT and in I in RCM) can be seen in the epidermis [Colour figure can be viewed at wileyonlinelibrary.com]

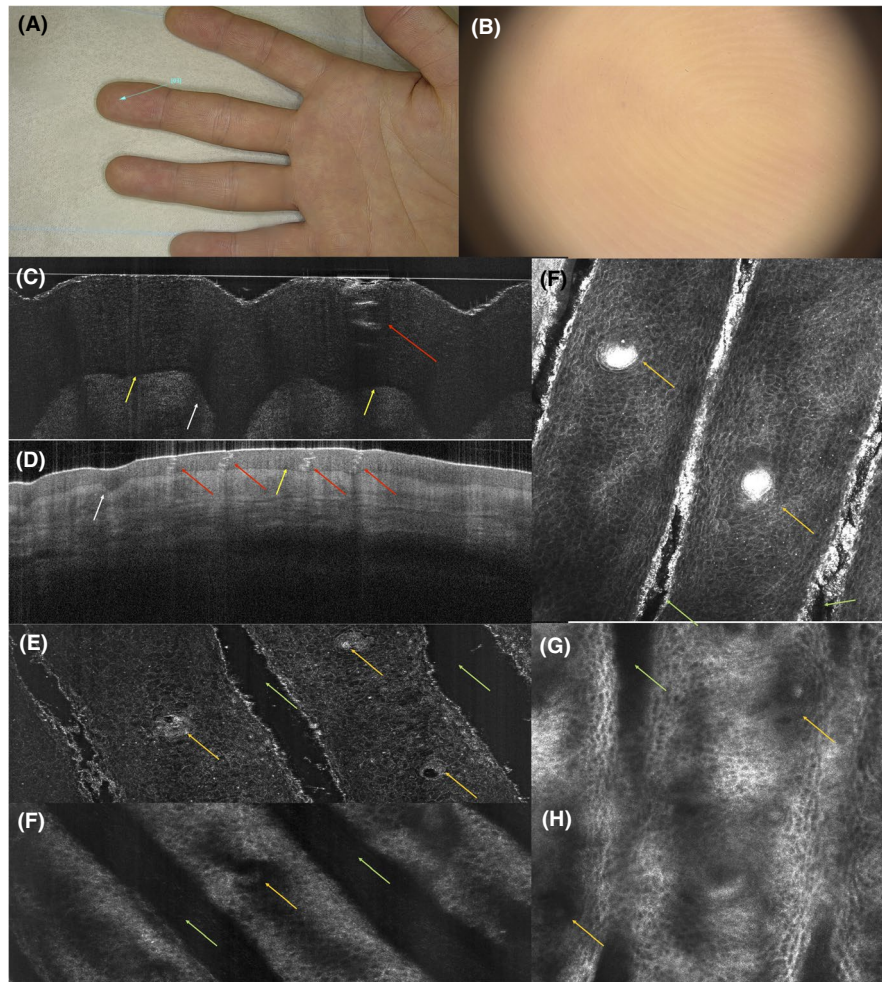


FIGURE 5 Comparison of en-face LC-OCT images and RCM images as well as vertical LC-OCT images and OCT images of the fingertip of the right index finger. (A and B) vertical LC-OCT images and in OCT images, a thick SC is typical for the fingertip, the entrance signal is very bright, and the surface has a characteristic wavy pattern. Hyperreflective, coiled eccrine ducts can be seen in the epidermis (red arrows in A and B in vertical LC-OCT images and in OCT images). A darker suprabasal line may correspond to the stratum lucidum (yellow arrows in A and B in vertical LC-OCT images and in OCT images). The DEJ is bright and follows the undulating pattern (white arrows in A and B in vertical LC-OCT images and in OCT images). The dermis cannot be effectively assessed at the fingertip due to the increased epidermal thickness and therefore limited penetration depth. In en-face mode, skin folds are very prominent dark furrows separating islands of polygonal keratinocytes (green arrows in C, E in LC-OCT images and D, F in RCM images). Roundish hyperreflective structures with a dark centre are regularly distributed among ridges of the skin marks and correspond to eccrine sweat ducts (orange arrows in C, E in LC-OCT images and D, F, G, H in RCM images) [Colour figure can be viewed at wileyonlinelibrary.com]

latter appears more rarefied and therefore darker in elderly skin (Figure 3C, Figure 4K in RCM and Figure 4L in LC-OCT) compared to the younger woman (Figure 4E in LC-OCT and Figure 4F in RCM). This can be especially visualized in the en-face mode. As signs of sun damage and therefore more represented in elderly skin, mottled pigmentation (red areas in Figure 4J in LC-OCT and in 4i in RCM) and hyperreflective tubular structures (solar lentigos) are seen in the epidermis and DEJ, respectively.

3.1.2 | Forearm

In en-face mode, SC was slightly thicker than on the face, showing a more irregular surface (Figure 2 vs. Figure 4). Numerous hair

follicles were visible, as well as dilated blood vessels in the PD (Figure 2C, E, G, I in LC-OCT images, Figure 2F, H and J in RCM images).

3.1.3 | Fingertip

In the vertical mode, a thick SC characterizes the fingertip (Figure 5A, B in vertical LC-OCT images and in OCT images) and, in general, the palmo-plantar skin. The entrance signal is very bright, and the surface has a characteristic wavy pattern (Figure 5A, B in vertical LC-OCT images and in OCT images). Hyperreflective, coiled eccrine ducts can be seen in the epidermis in continuity with the skin surface (red arrows in Figure 5A, B in vertical

LC-OCT images and in OCT images). The lower layers were less signal intense and corresponded to the stratum lucidum. The DEJ is bright and follows the undulating pattern. Due to the increased epidermal thickness and therefore limited penetration depth of the laser beam, the assessment of the dermis can be less effectively compared to other anatomical sites.

In en-face mode, skin folds are very prominent dark furrows separating islands of polygonal keratinocytes (green arrows in Figure 5C, E in LC-OCT images and Figure 5D, F in RCM images). Roundish hyperreflective structures with a dark centre are regularly distributed among ridges of the skin marks and correspond to eccrine sweat ducts (orange arrows in Figure 5C, E in LC-OCT images and Figure 5D, F in RCM images). Due to skin folds, the images at increasing depth show alternating, parallel layers of polygonal keratinocytes and junctional meshwork (corresponding to the meshwork junctional pattern in RCM terminology) interrupted by bright eccrine ducts.

3.1.4 | Basal cell carcinoma

In vertical mode in Figure 6, the BCC is clearly visible showing sharply demarcated tumour nests with palisading of the peripheral cells and surrounded by a dark cleft. This nodular BCC shows dark, cystic structures in the centre (yellow arrows in Figure 6D). The resolution is higher compared to OCT, where single cells cannot be distinguished. However, as in RCM, deeper BCC nests might not be identified and the lower border of the tumour is not visible. In en-face mode, slightly altered epidermal architecture with enlarged spaces between keratinocytes shows an atypical honeycombed pattern as in RCM (Figure 6F). Approaching the DEJ and the upper dermis, tumour nests (Figure 6G, I, J) appear both in LC-OCT and RCM as roundish or cord-like nests with peripheral palisading, surrounded by dark clefting and bright stromal reaction (Figure 6G, I). Canalicular blood vessels are also visible.

3.1.5 | Malignant melanoma (superficial spreading)

In vertical mode in Figure 7D, irregular conglomerates (red area) of bright ovoid cells (red arrows) corresponding to atypical melanocytes are visible at the DEJ, as an advantage compared to

OCT, which resolution does not allow a morphological distinction of melanomas or display of single cells. In Figure 7E) OCT can however provide useful information on the lesion like the tumour thickness and also about vascularization with the dynamic function.

In en-face mode, an atypical honeycombed pattern with altered keratinocytes of different size and shape can be distinguished in both LC-OCT (Figure 7G) and RCM (Figure 7J). The pagetoid spreading of melanoma cells is also visible, showing an invasion of large, hyperreflective round cells with big, dark nucleus and dendritic elements (Figure 7F, G, I, J). The structure of the DEJ is altered, showing irregular non-edged papillae both in RCM and LC-OCT. The PD often also shows irregular, fringed (coarse) collagen fibres, and a prominent vascularization. While RCM resolution for the characterization of single cells remains slightly better, nests of atypical cells and pagetoid spreading are clearly visible in LC-OCT, too.

3.1.6 | Actinic keratosis

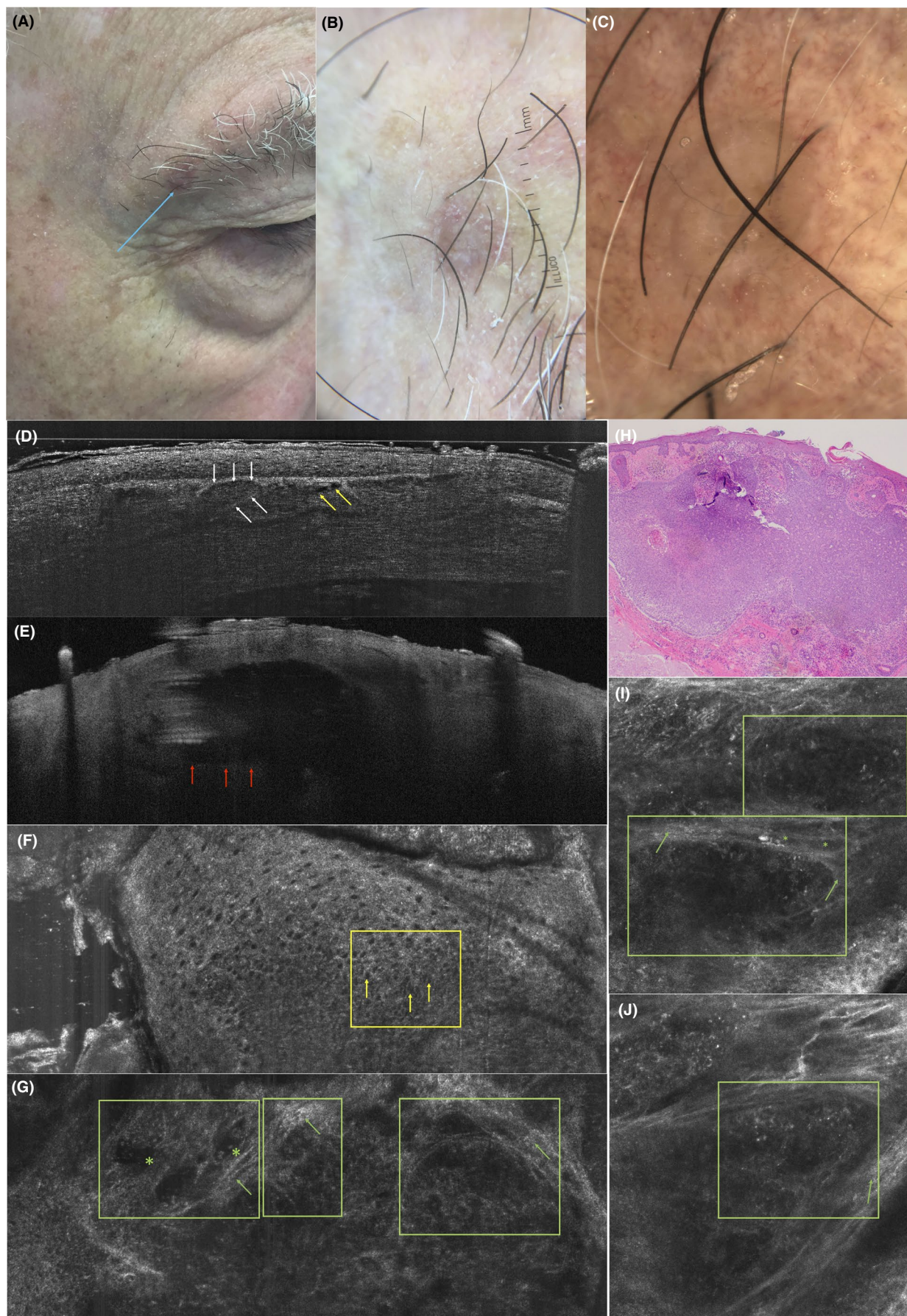
A pigmented actinic keratosis on the nose shows an irregular network of atypical keratinocytes in LC-OCT (Figure 8F, G, H). Individual cells are brighter due to pigmentation, of epithelioid shape (Figure 8G in LC-OCT, K in RCM). In contrast to lentigo maligna, a potential differential diagnosis, they are not grouped around the follicles. In OCT and in the vertical image of the LC-OCT, a thickening of the SC can be seen (Figure 8D in OCT, Figure 8E in LC-OCT). The changes in RCM (Figure 8J, K, L) with an atypical honeycomb pattern correspond to those of LC-OCT.

4 | DISCUSSION

In our experience, LC-OCT allows the visualization of single cells in both vertical and en-face mode. Vertical mode is directly comparable with OCT images and histological sections, while en-face mode is overlapping with RCM images and dermoscopy.

As expected, we could evaluate the stratum corneum (SC), the epidermis (EPI), the dermo-epidermal junction (DEJ) and the papillary dermis (PD) using LC-OCT, OCT and RCM. LC-OCT proved to have a higher penetration depth, allowing a more accurate

FIGURE 6 Basal cell carcinoma on the right eyebrow in en-face LC-OCT images and RCM images as well as vertical LC-OCT images and OCT images. (A) Clinical image of the basal cell carcinoma on the right eyebrow (blue arrow). (B and C) Dermoscopic images of the basal cell carcinoma on the right eyebrow (B) ILLUCO IDS-1100 (DermoScan GmbH, Regensburg, Germany and (C) VivaCam®, Mavig GmbH, Munich, Germany). (D and E): BCC is clearly visible as sharply demarcated tumour nests, with palisading of the peripheral cells and surrounded by a dark cleft (white arrows in Figure 6D, E). This nodular BCC shows dark, cystic structures in the centre (yellow arrows in Figure 6D). Deeper BCC nests, which can be seen in OCT, might not be identified in LC-OCT (red arrows in Figure 6E). In en-face mode, slightly altered epidermal architecture with enlarged spaces between keratinocytes shows an atypical honeycombed pattern as in RCM (yellow area with yellow arrows in Figure 6F). In the DEJ and the upper dermis, tumour nests (green areas in Figure 6G, I, J) appear both in LC-OCT and RCM as roundish or cord-like nests with peripheral palisading, surrounded by dark clefting and bright stromal reaction (green arrows in Figure 6G, I). Canalicular blood vessels are also visible (green asterisks in Figure 6G). (6H) Haematoxylin-eosin staining of the nodular cystic basal cell carcinoma on the right eyebrow (2 × magnification) [Colour figure can be viewed at wileyonlinelibrary.com]



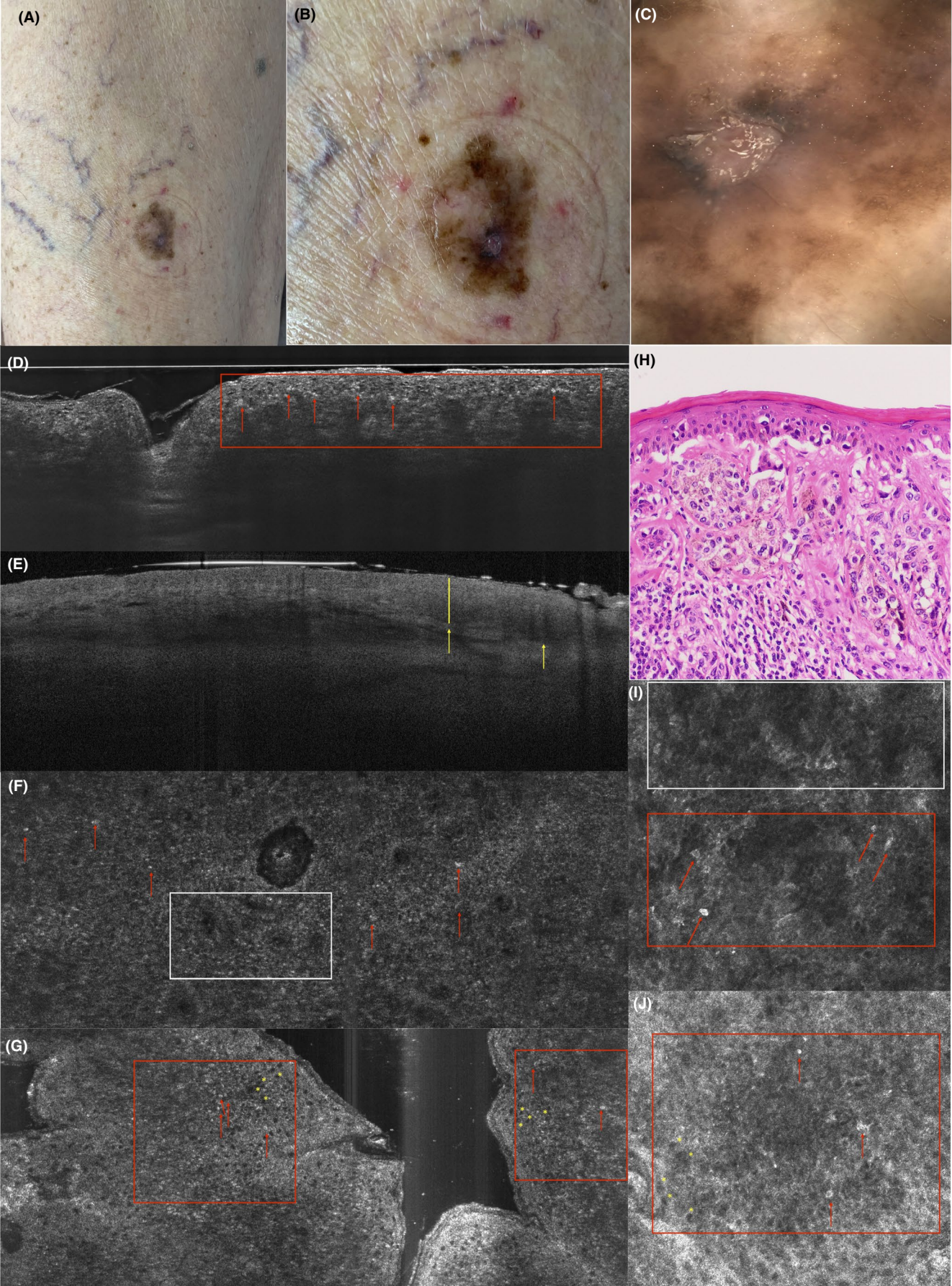


FIGURE 7 Superficial spreading malignant melanoma, tumour thickness 0.8 mm, on the right thigh in en-face LC-OCT images and RCM images as well as vertical LC-OCT images and OCT images. (A, B) Clinical images of the malignant melanoma on the right thigh. (C) Dermoscopic image of malignant melanoma on the right thigh (DermaGenius 2, DermoScan GmbH, Regensburg, Germany). In vertical mode in Figure D, irregular conglomerates (red area) of bright ovoid cells (red arrows) corresponding to atypical melanocytes are visible at the DEJ. In Figure E, OCT enables tumour thickness determination since the lower border is detectable. An atypical honeycombed pattern with altered keratinocytes of different size and shape (yellow asterisks) can be distinguished in both LC-OCT (in Figure 7G) and RCM (in Figure 7J). The pagetoid spreading of malignant melanocytes is also visible, showing an invasion of large, hyperreflective round cells with big, dark nuclei and dendritic elements (in Figure 7F, G, I, J red arrows). The structure of the DEJ is altered, showing irregular (non-edged) papillae both in RCM and LC-OCT (white areas). (H) Haematoxylin-eosin staining of the malignant melanoma on the right thigh (10 × magnification) [Colour figure can be viewed at wileyonlinelibrary.com]

evaluation of PD compared to RCM, a significant advantage for the characterization of, among others, collagen structures and tumour invasion. LC-OCT penetration depth is inferior to OCT (500 µm to about 1.5 mm); this feature can play an important role in the evaluation of the vascular plexus and neoangiogenesis as well as the thickness of invasive tumours, where deeper parts and the lower border can be missed by LC-OCT. The higher OCT penetration depth is however theoretical, since the effective resolution conspicuously decreases in the lower parts of the acquired images (dermis). The lateral resolution of OCT is not sufficient to distinguish single keratinocytes, so that only RCM and LC-OCT allow a cytological evaluation and assessment of atypia. Although the resolution of LC-OCT is slightly lower than RCM, an accurate acquisition free of motion artefacts allows the visualization of single cells, especially in the epidermis and in the en-face mode.

All three diagnostic devices allow a fast and non-invasive in vivo analysis of healthy skin and skin lesions at bedside. They are safe for use in children and pregnant women. They are delivered with a monitor, a mouse, a keyboard and a main computer unit connected to the probe with a cable. They are installed on a wheeled car that can be easily moved to enable bedside diagnostics.

While OCT does not need any interface, both RCM and LC-OCT require the use of immersion oil between the probe and the skin surface to reduce light reflection. Moreover, RCM needs a water-based immersion medium like ultrasound gel to reduce the spherical aberration of the laser beam passing through air and to ensure sufficient penetration of the light to the upper dermis.

Like ultrasound, all techniques are operator-dependent, so that the physician or technician is responsible for selecting the correct location to be analysed: the probe has to be kept stable during the examination in order to ensure a good quality of the acquisition in OCT, LC-OCT and RCM VivaScope® 3000. RCM VivaScope® 1500 requires a plastic window to be fixed to the skin, therefore it allows a more standardized image acquisition and accurate navigation thanks to the attached dermoscopic camera at the cost of a prolonged imaging time of some minutes. The newest version of Vivosight® OCT is provided with a camera, which makes an exact orientation within the lesion easier. A new LC-OCT device with a camera is scheduled for launching in spring 2020. The presence of a dermoscopic and/or clinical camera allows a more accurate examination of selected skin areas and lesions, decreasing the risk of misevaluations due to

unwanted relocation or slipping of the probe outside the target area.

Each device provides information about the actual scan depth. However, once the acquisitions have been exported as TIFF or JPEG, the depth is not visible in the single exported images except for RCM.

The short examination time allows the integration of all three devices into the daily routine. The authors observed a mean examination time of around 2-5 min for OCT and LC-OCT, while at least 10 min were needed for RCM due to the complexity of the software and the preparation of the probe.

The provided software is very intuitive in all three devices. The authors found the LC-OCT software the most intuitive and quickest (less than one minute to register a patient's record, lesion's location and launch the examination), followed by OCT (around a minute). There are however some software peculiarities for each device. LC-OCT is provided with additional 3D-slicer software, which reconstructs 3D cubes of an acquired stack of en-face images. OCT has extra software for an automated analysis of skin blood flow, skin texture and roughness (beta-version). Images can be easily exported as TIFF or JPEG with all devices. A global export and a single patient/lesion export are possible. An online learning platform and training courses are already available for OCT and RCM and planned for LC-OCT.

5 | CONCLUSIONS

The first lesions we measured with all three methods suggest that LC-OCT combines the advantages of RCM and OCT while reducing their disadvantages. LC-OCT seems to have optimal parameters regarding resolution and penetration depth to diagnose all types of skin cancer.

Larger systematic studies are needed to further characterize the field of use of this device and assess its sensitivity and specificity compared to histology.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

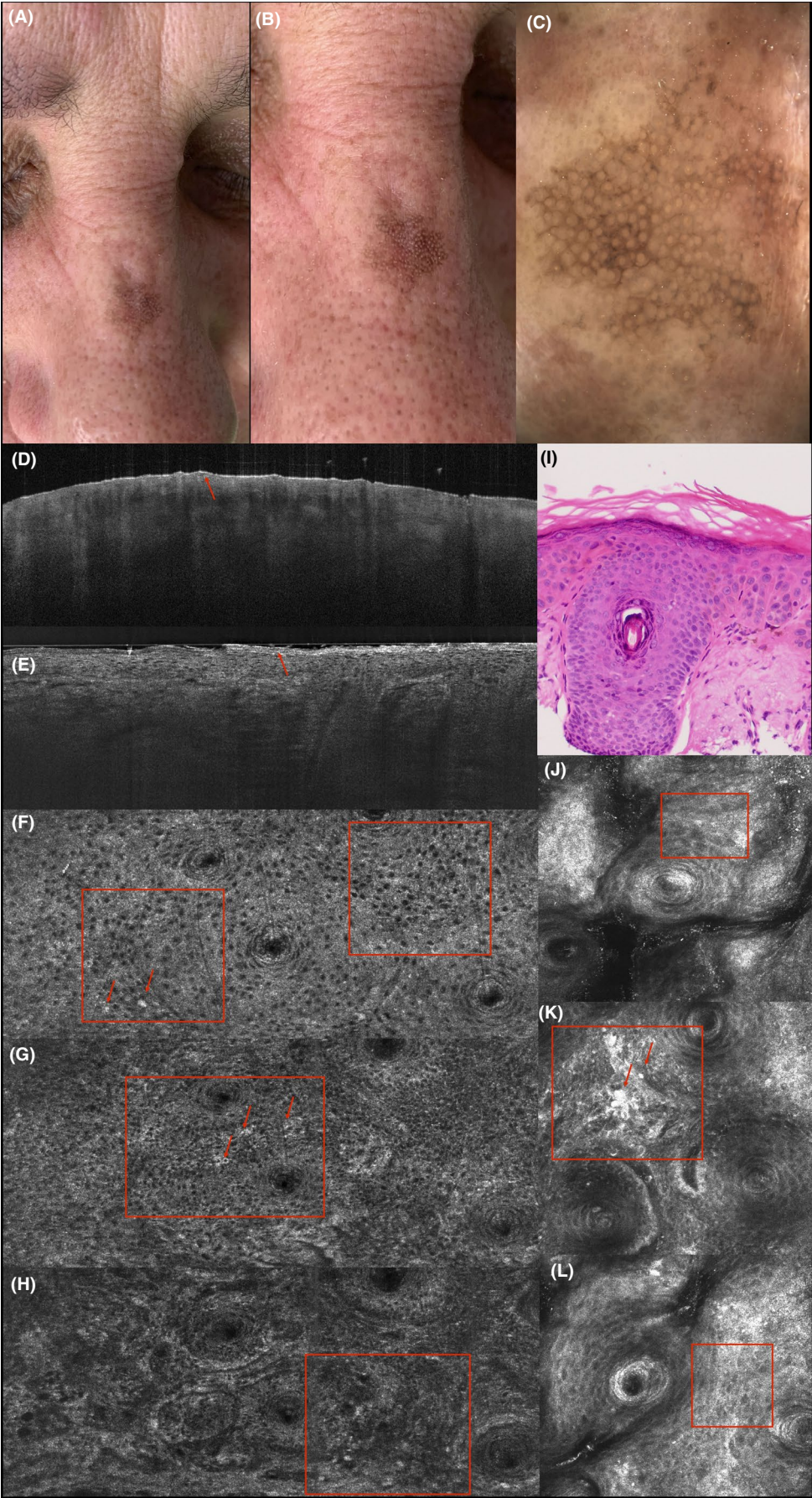


FIGURE 8 Pigmented actinic keratosis on the nose in en-face LC-OCT images and RCM images as well as vertical LC-OCT images and OCT images. (A and B) Clinical images of a pigmented actinic keratosis on the nose. (C) Dermoscopic images of a pigmented actinic keratosis on the nose (DermoGenius 2, DermoScan GmbH, Regensburg, Germany). (D and E) in OCT and in vertical LC-OCT images a thickening of the SC can be seen (red arrows). Figure 8F, G, H in LC-OCT and J, K, L in RCM images show an irregular network of atypical keratinocytes (red areas). Individual cells are brighter due to pigmentation of epithelioid shape and not dendritic (red arrows in Figure 8G in LC-OCT, K in RCM). They are not grouped around the follicles as in lentigo maligna, but are found interfollicularly. (I) Haematoxylin-eosin staining of the pigmented actinic keratosis on the nose (10 × magnification) [Colour figure can be viewed at wileyonlinelibrary.com]

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Article

In-Vivo LC-OCT Evaluation of the Downward Proliferation Pattern of Keratinocytes in Actinic Keratosis in Comparison with Histology: First Impressions from a Pilot Study

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Simple Summary: Actinic keratoses (AKs) are extremely common in the elderly population; they are universally recognized as precursors of invasive squamous cell carcinoma, and their risk of progression relates to the basal growth pattern of keratinocytes in the histological slides, based on a model called “PRO” classification. Since AKs can be investigated at bedside with non-invasive devices such the new line-field confocal optical coherence tomography (LC-OCT), we hypothesized that it was also possible to use such devices for reproducing the PRO classification and assessing the progression risk of AK without an invasive biopsy. In this pilot study, we demonstrated the feasibility of the LC-OCT severity grading of AKs based on the histological PRO classification, obtaining a good correlation between the two models and strong interobserver agreement.

Abstract: It is known that actinic keratoses (AKs) can progress to invasive squamous cell carcinoma (SCC). The histological PRO grading of AKs is based on the growth pattern of basal keratinocytes and relates to their progression risk. AKs can be non-invasively characterized by line-field confocal optical coherence tomography (LC-OCT). The aim of the study was to define criteria for an LC-OCT grading of AKs based on the PRO classification and to correlate it with its histological counterpart. To evaluate the interobserver agreement for the LC-OCT PRO classification, fifty AKs were imaged by LC-OCT and biopsied for histopathology. PRO histological grading was assessed by an expert consensus, while two evaluator groups separately performed LC-OCT grading on vertical sections. The agreement between LC-OCT and histological PRO grading was 75% for all lesions (weighted kappa 0.66, 95% CI 0.48–0.83, $p \leq 0.001$) and 85.4% when comparing the subgroups PRO I vs. PRO II/III (weighted kappa 0.64, 95% CI 0.40–0.88, $p \leq 0.001$). The interobserver agreement for LC-OCT was 90% (Cohen's kappa 0.84, 95% CI 0.71–0.91, $p \leq 0.001$). In this pilot study, we demonstrated that LC-OCT is potentially able to classify AKs based on the basal growth pattern of keratinocytes, in-vivo reproducing the PRO classification, with strong interobserver agreement and a good correlation with histopathology.

Keywords: actinic keratosis; PRO; dysplasia; keratinocyte cancer; field cancerization; line-field confocal optical coherence tomography; bedside histology; skin imaging; non-invasive diagnostics in dermatology

1. Introduction

Actinic keratoses (AKs) or solar keratoses are scaly, pink to reddish brown macules appearing as single to multiple elements on sun-exposed areas of elderly patients' skin. Clinically, AKs are divided into three main categories, from slightly palpable, to moderately thick, to hyperkeratotic [1].

Originally described as keratoma senilis, they were renamed by Hermann Pinkus, who combined their keratotic texture and their relationship with sunlight exposure [2]. Scalp, face, ears, neck, forearms and dorsal hands are the most involved body sites; males are more often affected than females [3]. In addition to UV radiation, other risk factors include fair skin, advanced age and chronic immunosuppression [4,5].

AKs are at risk of progressing to invasive squamous cell carcinoma (SCC) [6–11]. In fact, AKs and SCC share overlapping cytological features (atypical keratinocytes, loss of polarity, nuclear pleomorphism, altered maturation, higher mitotic index), which probably express the continuum of DNA damage due to chronic sun exposure, leading to the concept of field cancerization [12–15]. By definition, dysplastic alterations in AKs have to be confined to foci within the epidermis, while tumor strands invading the dermo-epidermal junction (DEJ) characterize invasive SCCs [16,17]. The most common histological classification divides AKs into three subgroups based on the distribution of the atypical keratinocytes within the epidermis, from the lower third (AK I), to two-thirds (AK II), to full thickness atypia (AK III) [9]. Surprisingly, this model does not correlate with progression to SCC, since the highest risk for invasion is instead associated with AKs with atypical keratinocytes restricted to the lower epidermis third (AK I). Recent studies observed that advanced basal proliferation of keratinocytes was related to progression to invasive SCC [18,19]. For this reason, a new AK histopathological classification focused on the basal growth pattern of keratinocytes was developed; the model was named “PRO I–III”, since it was focused on different stages of downward extension of basal keratinocytes, “protruding” into the papillary dermis. The earlier stage PRO I was characterized by the “crowding” of atypical keratinocytes in the basal layer, PRO II by their “budding” in round nests into the upper papillary dermis and PRO III by the “papillary sprouting”, with spikes of atypical keratinocytes protruding into the dermis and thicker than the overlying epidermis [19–21].

Multiple AKs often arise on sun-damaged and elderly skin, in the context of a field cancerization. Since it is not possible to predict which AKs will undergo progression into SCC through clinical examination, the whole field cancerization should be treated [22]. Non-invasive diagnostic technologies such as reflectance confocal microscopy (RCM), optical coherence tomography (OCT) and line-field confocal optical coherence tomography (LC-OCT) have been successfully used to enhance the diagnostic accuracy of AKs and to characterize the field cancerization in vivo [12,23–25].

As LC-OCT provides a cellular resolution, we hypothesized that it should be possible to in-vivo grade the basal growth pattern of AKs based on LC-OCT images. The aim of this study was to evaluate the agreement between the LC-OCT and histological PRO classification of AKs. A secondary aim was to assess the interobserver agreement in performing the LC-OCT PRO grading.

2. Materials and Methods

Fifty histologically confirmed AKs of the face, imaged with LC-OCT between January 2020 and 2021 at the Departments of Dermatology of the universities of Munich and Augsburg, were analyzed in the study. The lesions belonged to 26 males and 17 females, mean age 73.8 years (CI 57;86); we included the first sequentially recruited fifty lesions with a histologically confirmed diagnosis of AK and a complete set of evaluable LC-OCT images and histopathological slides. Previously treated and/or not histologically confirmed lesions were excluded a priori from the analysis. During the selection process, 5 additional histologically confirmed AKs were excluded before performing the evaluation and were therefore not included in the panel of the fifty lesions since the quality of LC-OCT acquisition was not sufficient to analyze the required parameters (availability of only

horizontal sections and not of vertical sections, artefacts such as oil bubbles). The whole lesion was imaged and analyzed with multiple LC-OCT scans, examining the epidermis, the DEJ and the dermis from the skin surface to a depth of approximately 500 μm . LC-OCT images sized $1.2 \times 0.5 \text{ mm}^2$ were acquired with a prototype device (DAMAE Medical, Paris), a class 1 supercontinuum laser with a central wavelength of 800 nm. Instrument and acquisition procedures have been described elsewhere [26,27]. Image acquisition was within a time frame of 0–7 days before the biopsy. The histologic specimens were 34 deep shave biopsies (with scalpel), 10 punch biopsies, 2 curettages (deep shave with ring curette) and 4 excisions. They were included in paraffin and stained with hematoxylin & eosin (H&E), according to the standard procedures, and evaluated as in daily routine examinations. Only completely evaluable tissue specimens with a vertical orientation were examined in the study.

We considered an H&E stained histological slide the gold standard. We did not perform any supplementary analysis with additional devices. PRO classification was made on both histological slides and LC-OCT vertical images based on the criteria of the original study by Schmitz et al. [21]: PRO I was defined by atypical keratinocytes crowded at basal epidermal layers (crowding), PRO II showed a slight protrusion into the upper papillary dermis with round nests of atypical keratinocytes (budding), thinner than the overlying epidermis, and PRO III displayed spikes of atypical cells thicker than the overlying epidermis (papillary sprouting). In the event that two or more patterns were present in the same image set, the higher score was chosen according to the literature [20,21]. LC-OCT images in vertical mode were evaluated, blinded to H&E slides, by a pair of dermatologists expert in non-invasive diagnostic techniques (observer round) and, separately, by a board-certified dermatopathologist/dermatologist and a dermatologist educated in dermatopathology, both experts in non-invasive diagnostic techniques (consensus round). In case of discrepancy regarding the grading, the dermatopathologist set the final decision, being the most experienced in the PRO classification. Hematoxylin & Eosin (H&E) stained slides were evaluated in a consensus by a board-certified dermatopathologist expert in both classification systems and a dermatologist educated in dermatopathology, blinded to LC-OCT images. Since the PRO classification was performed on histological slides in standard vertical sections, only vertical LC-OCT images were examined, while horizontal sections were not analyzed in order to avoid confounders and bias.

Cohen's weighted kappa coefficient with linear weights was used to calculate the correlation between LC-OCT and histopathological PRO score including confidence intervals (CI); the two cases labeled as indefinable with LC-OCT were excluded from the analysis. The correlation was also estimated into the different subgroups (PRO I and II/III). The interobserver agreement was calculated with Cohen's kappa coefficient, also taking into account the non-definable cases. A p -value lower than 0.05 was considered significant [28].

The study was approved by the ethical committee of the LMU Munich (Protocol Number 17–699).

3. Results

3.1. PRO Grading

Seventeen AKs were histologically classified as PRO I, 22 as PRO II and 11 as PRO III. LC-OCT PRO grading was defined as in Figure 1. The LC-OCT PRO grading was in agreement with the histological grading in 75% of the observations. The weighted kappa for LC-OCT and histological classification was 0.66 (95% CI 0.48 to 0.83; $p \leq 0.001$). Two cases were not classifiable with LC-OCT due to excessive lesion thickness. Discordant cases were either underestimated (12%) or overestimated (12%) (Table S1, Figure 2).

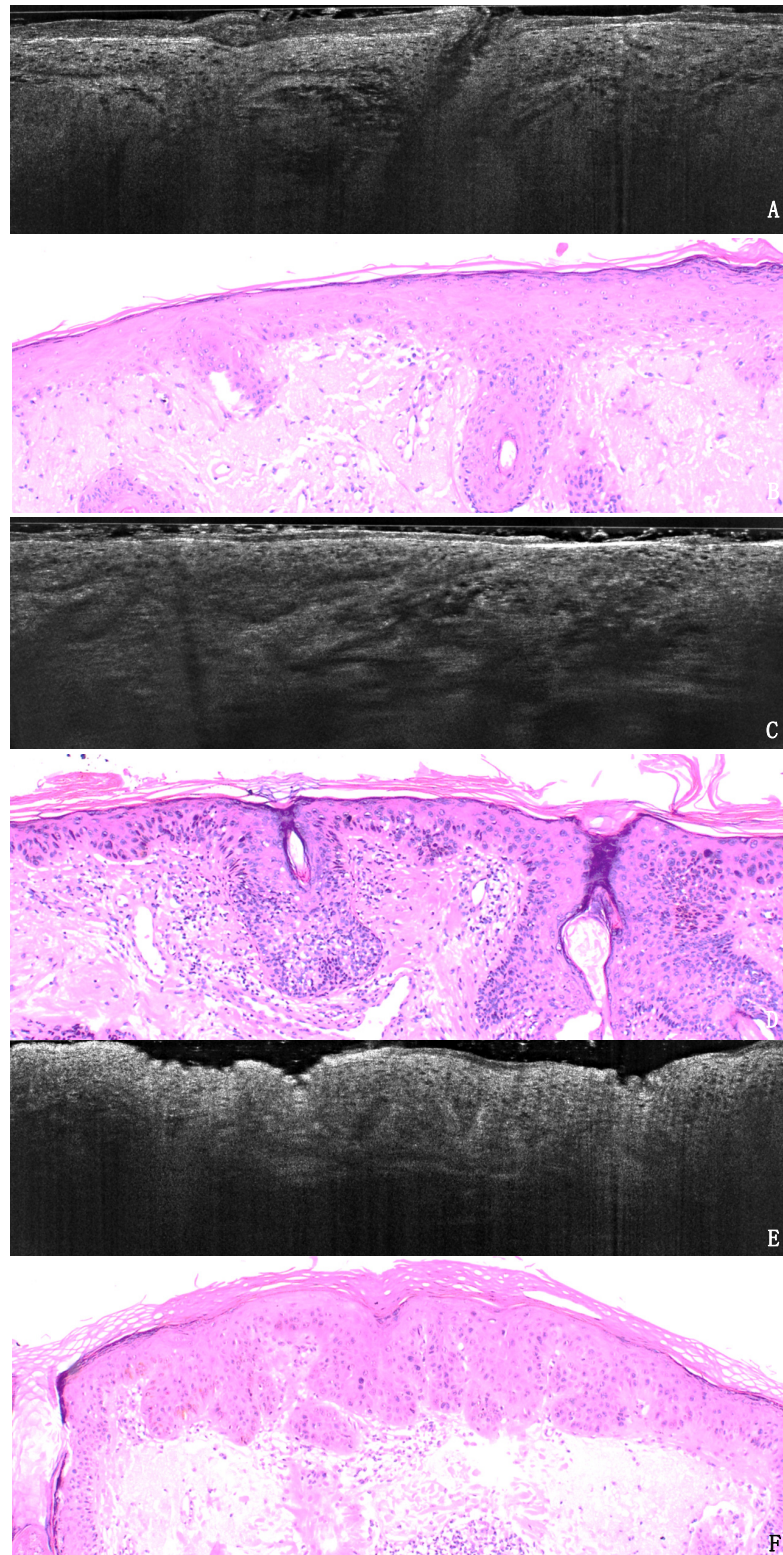


Figure 1. Example of three AK lesions in LC-OCT (A,C,E) and histology (B,D,F) in H&E sections, original magnification 40 $\times$. (A,B). AK PRO I shows basal crowding of keratinocytes in an otherwise regularly layered epidermis, with no protrusions into the dermis. (C,D). AK PRO II displays spikes of atypical keratinocytes slightly protruding into the papillary dermis. (E,F). AK PRO III shows the extended downward growth of atypical keratinocytes with spikes thicker than the overlying epidermis.

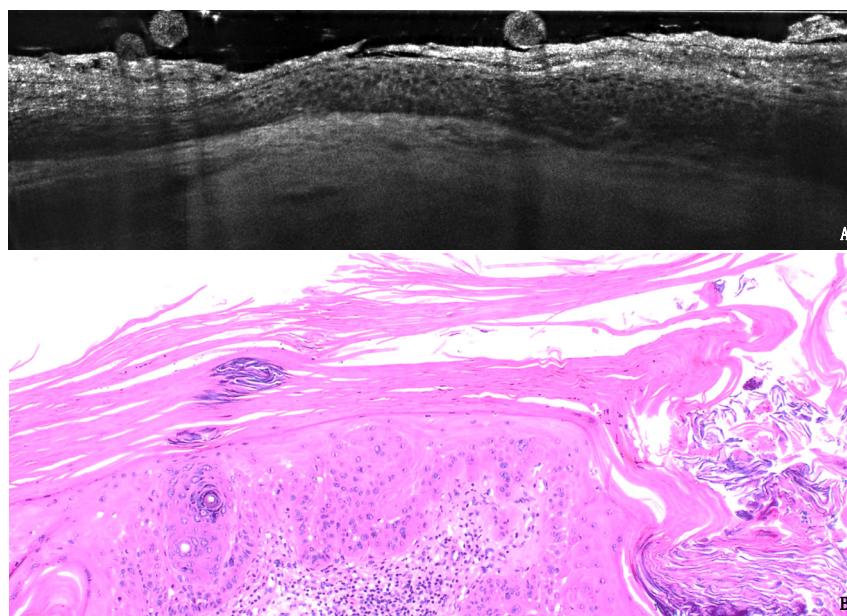


Figure 2. Example of AK lesion with discordant PRO grading between LC-OCT (A) and histology (40×, B): the hypertrophic AK on the scalp of an 85-year-old male patient was graded PROII in LC-OCT and PROIII in H&E, probably because of the diffuse hyperkeratoses impeding the evaluation of the deepest layers of the lesions.

If we take into account the subgroups, the agreement between LC-OCT and histology was 66.7% for PRO I, 86.5% for PRO II and 64% for PRO III. In the comparison between PRO I and PRO II-III grouped together as the higher progression risk category, the overall concordance was 85.4% (weighted kappa = 0.64, 95% CI, 0.40 to 0.88; $p \leq 0.001$) (Figure 3).

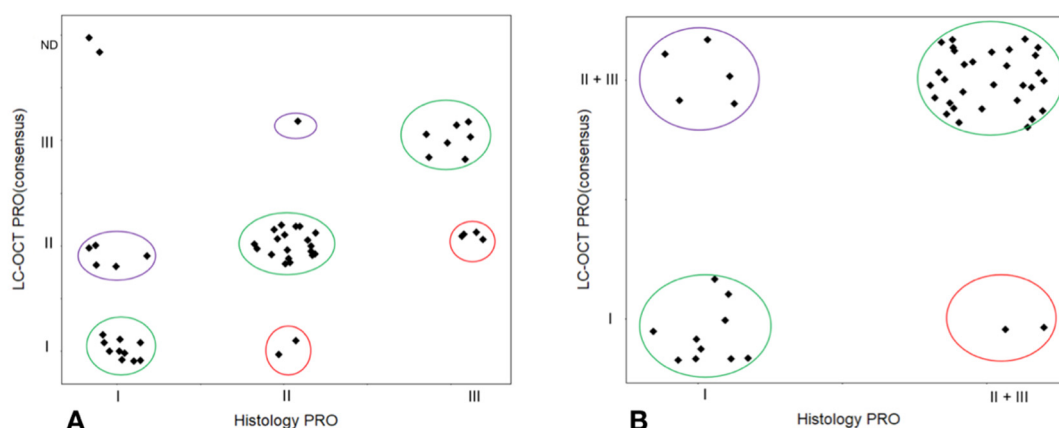


Figure 3. Overall (A) and subgroup (B) correlation between LC-OCT and histopathological PRO grading of AKs. ND: not definable.

3.2. Agreement

The interobserver agreement for LC-OCT PRO grading between the observer round and consensus round was 90%, with a Cohen's kappa = 0.84 (95% CI 0.71 to 0.97; $p \leq 0.001$). (Figure 4).

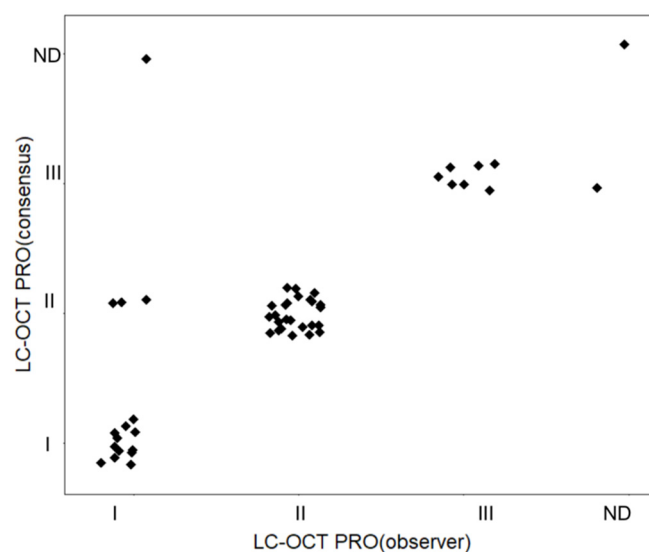


Figure 4. Interobserver agreement in the LC-OCT PRO classification of AKs. ND: not definable.

4. Discussion

AKs are one of the most common skin conditions in the elderly population, mainly arising on sun-exposed areas of the skin in the context of a field cancerization. Following the current guidelines, all AKs should be treated, since it is not possible to predict which ones will grow into an invasive SCC [29]. The type of treatment should be determined based on the clinical features, the patients' comorbidities and the previous therapies. Various local and field treatments, including surgery, laser ablation, photodynamic therapy and diverse topical treatments such as 5-fluorouracil, diclofenac, imiquimod and potassium hydroxide, can be used. It is known that diagnosis and follow-up of AKs can be improved by non-invasive diagnostic technologies such as RCM, OCT and LC-OCT, which provide a painless bedside examination of keratinocyte cancer (KC) [12,15,24,25,30–33]. RCM has been used for grading increasing keratinocyte dysplasia into different levels, with good correlation with histopathology, but it does not refer to specific classification systems [23].

We therefore focused on the in-vivo evaluation of the growth pattern of basal keratinocytes through LC-OCT, based on the recent PRO classification. The latter proved, in fact, to ensure the best interobserver agreement and the best risk stratification (in terms of progression risk to SCC) compared to other AK classifications [19–21]. We chose to use LC-OCT since it delivers dermoscopy-guided and real-time cross-sectional imaging of skin lesions with cellular resolution, so that the cytological and architectural features of the epidermis can be displayed in real time. It can visualize the epidermal layers, the DEJ and the superficial dermis, and its vertical acquisitions can be intuitively compared to histological slides [30,34,35]. Moreover, additional features such as perifollicular proliferation and adnexal extension can be visible, up to a depth of 500 μm . A direct and practical consequence is the potential in-vivo assessment of AK progression risk, since PRO III AKs are associated with a higher risk of developing an invasive SCC compared to PRO I [19–21].

In our study, we were able to visualize and grade the downward extension of AKs non-invasively through LC-OCT, defining key imaging patterns that can be used as reference images. We reported strong interobserver agreement in grading the basal growth pattern of keratinocytes in the examined AKs. Moreover, we reported good agreement in the PRO classification of AKs between LC-OCT and traditional histopathology. Additionally, we reached an overall agreement of 85.4% when comparing the subgroup of PRO I and the subgroup of PRO II/III AKs, which are the lesions at higher risk of progression to SCC compared to PRO I.

LC-OCT displays the protrusions of atypical basal keratinocytes into the upper dermis (spikes) and allows the measurement of their thickness compared to the overlying epidermis. This visual evaluation permits a reproducible LC-OCT grading of AKs based on the

PRO histological classification. We were able to classify PRO I as lesions with no visible spikes, PRO II as lesions with spikes slightly protruding into the papillary dermis and PRO III as lesions with more prominent spikes, thicker than the overlying epidermis (Figure 1, Figure 5). Our criteria can be used as a reference model for LC-OCT grading of AKs. The high reproducibility (90%) between observers demonstrates that skilled imaging experts are able to perform an in-vivo evaluation of cyto-architectural atypia with LC-OCT.

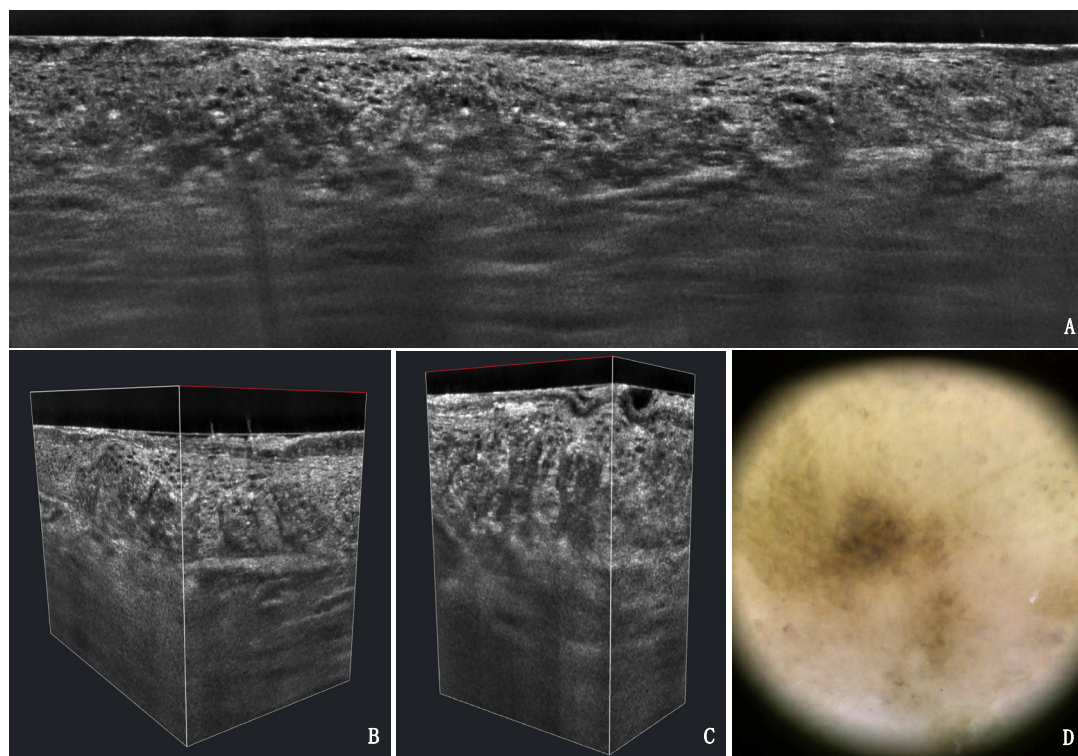


Figure 5. LC-OCT vertical (A), 3D (B,C) and dermoscopy (D) of a pigmented PRO III AK lesion on the dorsum of the hand of an 82-year-old male patient. The typical papillary sprouting as well as the pigmented basal keratinocytes are visible.

LC-OCT can be therefore considered a useful tool for the diagnosis and therapeutic monitoring of AKs and might be successfully used in both clinical trials and daily routine. As OCT and RCM, it can be used for a live, in-vivo mapping of several lesions in different progression stages and perilesional skin in the context of field cancerization, avoiding multiple surgical biopsies and related consequences (pain, discomfort, scars, inflammatory reactions, failed localization). Compared to conventional OCT, LC-OCT has a higher resolution and allows the assessment of cytological features and not only structural details. Compared to RCM, LC-OCT displays vertical sections simultaneously to horizontal slides as in RCM. Thus, it is much easier in LC-OCT images to determine the degree of basal proliferation and compare it with histology.

Some limitations have to be taken into account. In our study, two large and thick hyperkeratotic lesions were not fully evaluable. This sheds light on the good but still not perfect penetration depth of the LC-OCT, which does not fully display the basal layer in the presence of severe hyperkeratosis. In such cases, it is useful to examine the margins of the lesion, since the basal growth pattern of keratinocytes does not depend on the lesion thickness. Moreover, in this regard, it was easier for the observers to evaluate the downward growth pattern of basal keratinocytes in the context of a PRO classification, compared to the distribution of the atypical keratinocytes through the epidermis, as in other classifications. Another limitation of the study was the small sample size (50 lesions, belonging to 43 patients). Since some lesions arose on the same patient, a bias derived from this aspect cannot be excluded; however, we only included independent lesions, arising on

different anatomical sites and separately imaged and biopsied, so that we considered the number of cases acceptable for a lesion-centered, pilot study [36]. An additional, crucial point that has to be taken into account is represented by the observer's experience, not only in non-invasive diagnostic techniques but also in dermatopathology. All observers in this study had knowledge of dermatopathology and non-invasive imaging; it was not possible to perform a correlation of the LC-OCT grading and the level of experience of the observer.

The agreement between LC-OCT and histology was lower in PRO I compared to PRO II/III, due to overestimation. Five cases graded as PRO I in histology were evaluated as PRO II/III with LC-OCT. This might be due to confounders such as hair follicles and shafts, which might appear as longitudinal structures in the sections, but also to the larger field of view, providing a global analysis of the examined area. LC-OCT can, in fact, scan the whole AK lesion in a three-dimensional way, guided by a camera, displaying multiple scans; on the contrary, the pathologist only has a few histological sections at their disposal, since only a few slides are obtained from each tissue block in the daily diagnostic routine. From this perspective, LC-OCT might provide additional details regarding non-invasive AK grading, correctly directing lesions to either conservative or surgical therapy based on their basal growth pattern.

5. Conclusions

LC-OCT is able to perform a reliable in-vivo evaluation of basal keratinocyte growth pattern based on the histopathological PRO classification of AKs, with strong agreement with histopathology and between different observers. Larger studies, also potentially aided by artificial intelligence algorithms, are needed to validate the method.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/cancers13122856/s1>, Table S1: PRO grading evaluated with LC-OCT in the observer round, consensus round and histopathology.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Line-field confocal optical coherence tomography for the in vivo real-time diagnosis of different stages of keratinocyte skin cancer: a preliminary study

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Abstract

Background The treatment of keratinocyte cancers (KC) strictly depends on their differentiation and invasiveness. Non-invasive diagnostic techniques can support the diagnosis in real time, avoiding unnecessary biopsies. This study aimed to preliminarily define main imaging criteria and histological correlations of actinic keratosis (AK), Bowen's disease (BD) and squamous cell carcinoma (SCC) using the novel device line-field confocal optical coherence tomography (LC-OCT).

Methods Dermoscopy and LC-OCT images of 73 histopathologically confirmed lesions (46 AKs, 11 BD and 16 SCCs) were included in the study. Exemplary lesions (10 AKs, 5 BD and 5 SCCs) were additionally investigated with optical coherence tomography and reflectance confocal microscopy.

Results Most common LC-OCT findings of KC in the descriptive statistics were hyperkeratosis/parakeratosis, disruption of stratum corneum, broadened epidermis, basal and suprabasal keratinocyte atypia, dilated vessels/neoangiogenesis and elastosis/collagen alterations. In the univariate multinomial logistic regression, a preserved DEJ was less common in SCC compared with AK and BD, BD displayed marked keratinocyte atypia involving all epidermal layers (bowenoid pattern), while SCC showed ulceration, increased epidermal thickness, keratin plugs, acantholysis, not visible/interrupted DEJ and epidermal bright particles. LC-OCT increased the diagnostic confidence by 24.7% compared with dermoscopy alone.

Conclusions Our study describes for the first time specific LC-OCT features of different stages of KC and their histopathological correlates, focusing on keratinocyte morphology and architecture of the epidermis and DEJ. LC-OCT may open new scenarios in the bedside diagnosis, treatment planning and follow-up of KC.

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Conflicts of interest

None declared.

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Introduction

Keratinocyte skin cancer (KC), from its early stages of actinic keratosis (AK) and Bowen's disease (BD) to invasive squamous cell carcinoma (SCC), belongs to the most common tumours in

elderly patients, related to chronic solar damage or immunosuppression.¹

In order to prevent the transformation from subclinical keratinocyte dysplasia to invasive SCC and to lower the social and economic burden of the disease, early detection and treatment are essential.²

The diagnosis of KC is based on clinical and dermoscopic examination, eventually followed by a biopsy.¹ Dermoscopy has a good reported sensitivity and specificity in diagnosing and

[†]The authors share their senior authorship.

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grading KC, although significantly higher for AKs (51.2%–98%; 95%) rather than SCCs (55%–79%; 84%–87%) and mainly calculated in the context of retrospective studies, so that further prospective studies are needed.^{3–11} Non-invasive diagnostic methods such as reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) have been widely used in the diagnosis of AKs^{12–16}, while few articles analysed the sensitivity and specificity of such tools for invasive SCCs.^{14,17} RCM, which displays horizontal sections, allows a detection of characteristic AK patterns with high resolution^{10,18}; however, its low penetration depth (up to 200–250 μm) can be a limit in evaluating the DEJ and the infiltration of the dermis in thick lesions. On the contrary, OCT does not permit a clear evaluation of cellular morphology in its vertical sections but is able to detect invasive lesions thanks to its higher penetration depth (up to 1.5 mm), and can display even their vascularity in dynamic mode.¹⁹

The new device line-field confocal OCT (LC-OCT) creates scans at higher resolution than conventional OCT, while reaching a higher detection depth compared to RCM, and can potentially be used for a non-invasive diagnosis of KC and several other skin diseases.^{15,20–27} To date, no systematic studies on this topic have been published, so that we aimed to define main LC-OCT criteria for the diagnosis of KC, based on the histological criteria and the ones already described for similar diagnostic techniques. In addition, we preliminarily compared LC-OCT images with OCT and RCM to exemplarily point out the differences, strengths and limitations of the methods.

Materials and methods

Clinical, dermoscopic (FotoFinder GmbH, Germany and DermoGenius-DermoScan GmbH, Germany) and LC-OCT images of suspected KC were prospectively collected and analysed at the University of Munich and Augsburg. Cases were analysed by three imaging experts blinded to the histological diagnosis in a consensus, with an almost perfect agreement. In discordant cases, three more experts were involved for consensus. Only histopathologically confirmed lesions with a complete image set were included in the analysis. LC-OCT features were defined based on main histological parameters and the nomenclature chosen based on the current histology, OCT and RCM terminology. We chose not to define a novel terminology because LC-OCT allows a very intuitive comparison with the corresponding histological features because of its cellular resolution and visualization of vertical slices. Following criteria were investigated: epidermal thickness in μm (obtained with ten repeated measurements taken every 120 μm on the vertical image of the 1.2 mm long LC-OCT field of view in the centre of the lesion using the incorporated software tool), ulceration, disruption of stratum corneum (SC), hyperkeratosis, compact parakeratosis, scales, keratin plugs, thinning of the epidermis, broadened/acanthotic epidermis, suprabasal and basal atypical keratinocytes, bowenoid pattern, acantholysis, pyknotic cells, atypical honeycombed pattern, bright particles in the

epidermis, dendritic cells, flattened rete ridges, well-defined dermo-epidermal junction (DEJ), not visible/interrupted DEJ, dilated vessels/neoangiogenesis, glomerular vessels, bright particles in the dermis and elastosis/collagen alterations. Keratinocytes were defined as atypical when visually markedly differing from each other in size, shape and contours compared with healthy skin. We defined the thickened, acanthotic epidermis with roundish ('bowenoid') contours built by enlarged atypical keratinocytes in all epidermal layers as 'bowenoid pattern'.

The level of diagnostic confidence was defined on a three-point scale from 0% to 100% as follows: high (>70%), average (51%–69%) and poor (<50%). LC-OCT images were acquired with a prototype device (DAMAE Medical, Paris), a class 1 supercontinuum laser with a central wavelength of 800 nm, with three imaging modalities: vertical and horizontal ($1.2 \times 0.5 \text{ mm}^2$) and 3D ($1.2 \times 0.5 \times 0.5 \text{ mm}^3$). Instrument and acquisition procedures were described elsewhere.^{21,28} Exemplary lesions (10 AKs, 5 BD and 5 SCCs) were imaged with a conventional OCT (VivoSight, Michelson-Diagnostics, UK) and RCM (VivaScope 1500–3000, Mavig, Germany) device.

Statistical analysis

For descriptive statistics, mean values with 95% confidence interval were calculated for numeric data, while absolute numbers with percentage portion were given for nominal data. To evaluate the discriminant value of LC-OCT features for the distinction between AK, BD and SCC, univariate multinomial logistic regressions were performed for each individual feature. Odds ratios (OR) with corresponding *P*-values representing the effect of one feature on the likelihood of a diagnosis being made were calculated for: (1) AK vs. BD and (2) BD vs. SCC (3) AK vs. SCC.

We additionally included all predictors that showed a significant OR in a multivariable multinomial logistic regression analysis, as done by Peppelman *et al.*²⁹ to estimate the correct classification rate that is reached by using all information contained in the predictors showing significance in the univariate multinomial logistic regression analyses. We excluded the results of this analysis from our results because of overfitting.

P-values of less than 0.05 were considered statistically significant. All statistical analyses were conducted using R (version 3.6.0, 2019, R Foundation for Statistical Computing).

All patients provided their written informed consent. The study was approved by the local ethics committee (No. 17-699).

Results

Population

123 lesions suspicious for KC were recruited prospectively of which 73 cases were enrolled after histopathologic confirmation (belonging to 25 females, 48 males, mean age 74.8 years, CI 72.7–76.9). Remaining cases were excluded due to missing histopathological confirmation of the diagnosis as gold standard. Forty-six

AKs (10 hypertrophic, 5 atrophic, 3 bowenoid), 11 BD and 16 invasive SCCs were analysed. Most common lesion site was the head and neck area (52.1% face, 28.8% scalp), followed by upper limbs (9.6%), lower limbs (5.5%), trunk (2.7%) and genital area (1.4%).

Diagnostic confidence

Our diagnostic confidence with LC-OCT was high in 74% and average in 26% of the cases; with dermoscopy, it was high in 49.3% and average in 49.3%. We therefore estimated an improvement of 24.7% compared with clinical examination and dermoscopy alone.

Morphological features

The descriptive statistics and the univariate multinomial logistic regressions of the dermoscopic features are available in Tables S1 and S2.

Main LC-OCT features of AKs in vertical mode as reported in the descriptive statistics were (Tables 1 and 2) (Figures 1 and 2, S1): hyperkeratosis with compact parakeratosis (95.7% and 67.1%, respectively), scales (67.4%), broadened acanthotic epidermis (68.2%) and keratinocyte atypia (95.5%). It was not always possible to exactly distinguish between basal and suprabasal layers. The DEJ was preserved in 82.6%. The dermis showed dilated vessels/neoangiogenesis (59.5%) and elastosis/collagen alterations (56.1%). BD was characterized by a marked keratinocyte atypia involving all epidermal layers (100%) but with a well-defined DEJ (80%) (Figures 1 and 3, S2). In 7 AKs and 2 BD, some parts of the DEJ were not visible.

In invasive SCC, hyperkeratosis (100%), ulceration (68.8%) and disruption of SC (100%), a marked keratinocyte atypia (100%) in a broadened epidermis (93.8%) was found, with an

Table 1 Descriptive statistics of LC-OCT features of actinic keratosis, Bowen's disease and squamous cell carcinoma evaluated in the study with their relative and absolute frequencies

Features	All subtypes		Actinic keratosis		Bowen's disease		Invasive squamous cell carcinoma	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Epidermal thickness	155.8	134.0, 177.5	126.2	102.9, 149.5	168.5	107.1, 229.8	232.0	182.3, 281.7
	%	n	%	n	%	n	%	n
Epidermis								
Ulceration	23.9	17/71	6.8	3/44	27.3	3/11	68.8	11/16
Disruption of stratum corneum	82.2	60/73	71.7	33/46	100.0	11/11	100.0	16/16
Hyperkeratosis	97.3	71/73	95.7	44/46	100.0	11/11	100	16/16
Compact parakeratosis	83.6	61/73	67.1	35/46	100.0	11/11	93.8	15/16
Scales	78.1	57/73	67.4	31/46	90.9	10/11	100.0	16/16
Keratin plugs	50.7	37/73	39.1	18/46	54.5	6/11	81.2	13/16
Thinning of the epidermis	28.2	20/71	40.9	18/44	18.2	2/11	0.0	0/16
Broadened/acanthotic epidermis	78.9	56/71	68.2	30/44	100.0	11/11	93.8	15/16
Suprabasal atypical keratinocytes	71.2	52/73	54.3	25/46	100.0	11/11	100.0	16/16
Basal atypical keratinocytes	94.5	69/71	95.5	42/44	100.0	11/11	100.0	16/16
Bowenoid pattern	27.8	20/72	15.2	7/46	90.0	9/10	25.0	4/16
Acantholysis	23.2	16/69	15.9	7/44	20.0	2/10	46.7	7/15
Pyknotic cells	43.1	31/72	32.6	15/46	20.0	5/10	68.8	11/16
Atypical honeycombed pattern	97.2	70/72	95.7	44/46	100.0	10/10	100.0	16/16
Bright particles in the epidermis	31.4	22/70	25.0	11/44	10.0	1/10	62.5	10/16
Dendritic cells	5.7	4/70	4.5	2/44	20.0	2/10	0	0/16
Flattened rete ridges	56.5	39/69	65.1	28/43	5.5	6/11	33.3	5/15
Dermo-epidermal junction								
Well defined	68.1	49/72	82.6	38/46	80.0	8/10	18.8	3/16
Not visible/interrupted	28.8	21/73	15.2	7/46	36.4	4/11	81.2	13/16
Dermis								
Dilated vessels/neoangiogenesis	65.2	43/66	59.5	25/42	70.0	7/10	78.6	11/14
Glomerular vessels	13.6	9/66	9.5	4/42	20.0	2/10	21.4	3/14
Bright particles in the dermis	18.8	13/69	11.4	5/44	20.0	2/10	40.0	6/15
Elastosis/collagen alterations	61.9	39/63	56.1	23/41	70.0	7/10	75.0	9/12

CI, confidence interval; %, per cent (relative frequency); n, number (absolute frequency).

Table 2 Univariate multinomial logistic regression reporting odds ratios (OR) and *P*-values of main analysed LC-OCT parameters

Feature LC-OCT	Subtype	OR	<i>P</i>
Epidermal thickness	2 1	1.01	0.13
	3 1	1.01	<i>P</i> < 0.001***
	3 2	1.01	0.10
Ulceration	2 1	5.12	0.07050
	3 1	30.06	<i>P</i> < 0.001***
	3 2	5.87	<i>P</i> < 0.05*
Disruption of stratum corneum	2 1	5548.60	0.81
	3 1	9400.90	0.81
	3 2	15.70	0.98
Hyperkeratosis	2 1	978.45	0.88
	3 1	896.47	0.85
	3 2	12.36	0.98
Compact parakeratosis	2 1	2212.53	0.76
	3 1	4.72	0.15
	3 2	0.00	0.84
Scales	2 1	4.80	0.15
	3 1	8192.20	0.78
	3 2	1509.60	0.81
Keratin plugs	2 1	1.87	0.36
	3 1	6.74	<i>P</i> < 0.01**
	3 2	3.61	0.15
Thinning of the epidermis	2 1	0.32	0.18
	3 1	0.00	0.80
	3 2	0.00	0.85
Broadened/acanthotic epidermis	2 1	5689.80	0.80
	3 1	7.00	0.07
	3 2	0.00	0.99
Suprabasal atypical keratinocytes	2 1	12044.20	0.79
	3 1	20060.40	0.80
	3 2	12.60	0.99
Basal atypical keratinocytes	2 1	923.79	0.87
	3 1	867.28	0.84
	3 2	12.87	0.99
Bowenoid pattern	2 1	50.14	<i>P</i> < 0.001***
	3 1	1.86	0.38
	3 2	0.04	<i>P</i> < 0.05**
Acantholysis	2 1	1.32	0.75
	3 1	4.63	<i>P</i> < 0.05*
	3 2	3.50	0.18
Pyknotic cells	2 1	2.08	0.30
	3 1	4.55	<i>P</i> < 0.05*
	3 2	2.20	0.34
Atypical honeycombed pattern	2 1	1687.40	0.90
	3 1	3545.80	0.91
	3 2	12.4	0.98
Bright particles in the epidermis	2 1	0.33	0.32
	3 1	5.00	<i>P</i> < 0.01**
	3 2	15.00	<i>P</i> < 0.05*
Dendritic cells	2 1	5.250	0.12
	3 1	0.00	0.88
	3 2	0.00	0.84

Table 2 Continued

Feature LC-OCT	Subtype	OR	<i>P</i>
Flattened rete ridges	2 1	0.64	0.52
	3 1	0.27	<i>P</i> < 0.05*
	3 2	0.42	0.28
Well-defined DEJ	2 1	0.84	0.85
	3 1	0.049	<i>P</i> < 0.001***
	3 2	0.058	<i>P</i> < 0.01**
Not visible/interrupted DEJ	2 1	3.18	0.12
	3 1	9.28	<i>P</i> < 0.001***
	3 2	2.92	0.19
Dilated vessels/neoangiogenesis	2 1	1.59	0.54
	3 1	2.49	0.21
	3 2	1.57	0.63
Glomerular vessels	2 1	2.38	0.36
	3 1	2.59	0.26
	3 2	1.09	0.93
Bright particles in the dermis	2 1	1.95	0.47
	3 1	5.20	<i>P</i> < 0.05*
	3 2	2.67	0.30
Elastosis/collagen alterations	2 1	1.83	0.43
	3 1	2.35	0.25
	3 2	1.29	0.79

Nomenclature: 1 = actinic keratosis, 2 = Bowen disease, 3 = squamous cell carcinoma. Abbreviations: DEJ, dermo-epidermal junction; OR, odds ratio; Significance levels: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

either interrupted or non-visible DEJ (81.2%). (Figures 1 and 4). Dilated vessels/neoangiogenesis (78.6%) and collagen alterations (75%) were noticed.

In horizontal sections, SC was disrupted in 71.7% of AKs and 100% of SCCs; an atypical honeycombed pattern as described in RCM¹³ was found in 95.7% of AKs and 100% of SCCs, pyknotic (broken) cells in 32.6% AKs, 68.8% SCCs. Collagen alterations and dilated vessels/neoangiogenesis were seen in all types of lesions; in 90% of BD, glomerular vessels (the typical tortuous, dilated vessels inside the dermal papillae) were displayed (Figure 3).

Mean epidermal thickness was 126.2 µm in AKs, 168.5 µm in BDs and 232 µm in SCCs (Table 1).

Univariate multinomial logistic regression models were performed to identify the main LC-OCT patterns allowing the distinction between different entities (AK, BD and SCC).² Following features were helpful in distinguishing SCCs compared to AKs: increased epidermal thickness (*P* < 0.001), ulceration (*P* < 0.001), keratin plugs (*P* < 0.001), acantholysis (*P* < 0.05), bright particles in the epidermis (*P* < 0.01), flattened rete ridges (*P* < 0.05), not visible/interrupted DEJ (*P* < 0.001) and bright particles in the dermis (*P* < 0.05). Ulceration (*P* < 0.05) and bright particles in the epidermis (*P* < 0.05) could analogously help differentiate a SCC from a BD, while the OR for BD well-defined DEJ (*P* < 0.01) and bowenoid pattern

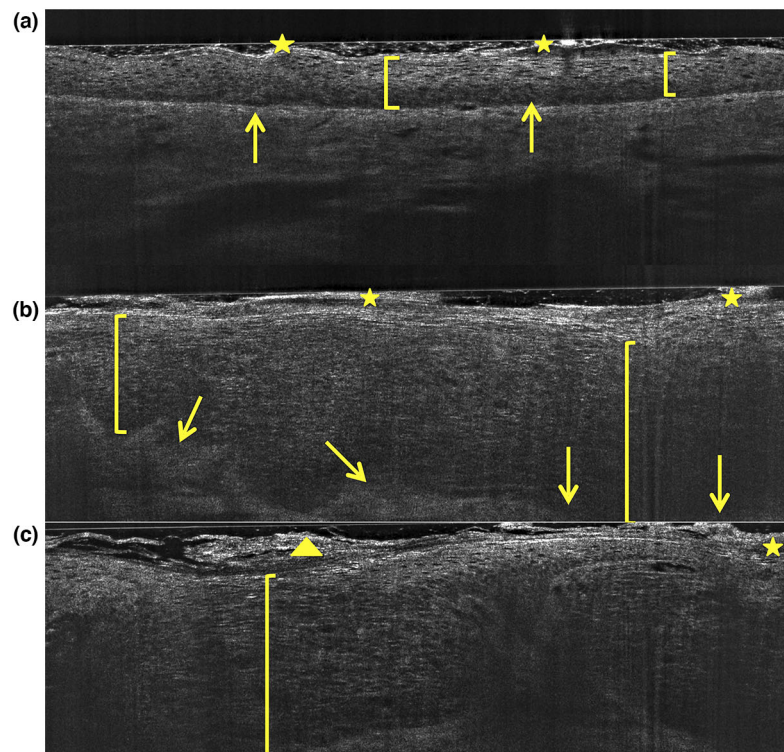


Figure 1 Different stages of keratinocyte skin cancer in LC-OCT, vertical mode: (a) AK of the face, (b) BD of the back, (c) SCC of the scalp. Hyperkeratosis and parakeratosis (star), atypical keratinocytes (bracket) are seen in all lesions; the DEJ (arrow) is preserved in (a) and (b), but not in (c), where strands of atypical keratinocytes infiltrating the dermis are seen (bracket) together with a thick, hyperkeratotic crust (triangle). AK, actinic keratosis; BD, Bowen disease; DEJ, dermo-epidermal junction; LC-OCT, line-field confocal optical coherence tomography; SCC, squamous cell carcinoma.

($P < 0.01$) was significantly higher for BD compared to SCC. The bowenoid pattern was the distinguishing feature of BD compared to AKs ($P < 0.001$).

Discussion

Differentiating stages of field cancerization noninvasively in real time is of outmost importance in the clinical practice since it allows an efficient therapy planning.

A dermoscopic 'progression model' was developed by Zalaudek *et al.*, who described a red pseudonetwork ('strawberry pattern') as typical for early AKs, and a red starburst pattern and yellow-white scales as a sign of transition to intraepidermal carcinoma. In BD, increasing neovascularization is seen as clustered glomerular vessels, while scales tend to become thicker and centrally coalesce. Advanced SCCs are characterized by irregular vessels, increased keratinization and ulceration.³ Our findings were in line with the common literature on the topic: glomerular vessels were the most significant finding in dermoscopy of BD, while starburst pattern, scales and ulceration were seen in SCCs.

Conventional OCT, which provides vertical images intuitively comparable to histological sections and LC-OCT vertical mode, is able to detect common features of KC such as hyperkeratosis, epidermal disruption and loss of the DEJ. However, hyperkeratosis affects the imaging of underlying structures and an exact discrimination of the invasiveness is not always possible.^{14,19,30–32} In their systematic review, Friis¹⁹ described most common OCT features of AKs: disruption of layers, white streaks and dots and thickened epidermis. Schuh and Welzel demonstrated on a very large sample that AKs can be easily differentiated from normal skin and BCCs thanks to the decreased local signal intensity, as well as thicker SC and epidermis, finding moreover very good correlations between OCT and histopathological thickness of the skin layers.³³ In a paper focused on differentiation of non-melanoma skin cancers (NMSC), Batz *et al.* associated multifocal acanthosis to Bowen's disease and SCC, with SCC additionally showing hyperreflective nests and non-compressible dark borders.³⁴ No systematic study on OCT-guided diagnosis of SCC was published. Yet high-definition OCT (not commercially available anymore) was also used by Boone *et al.* for the in vivo discrimination of

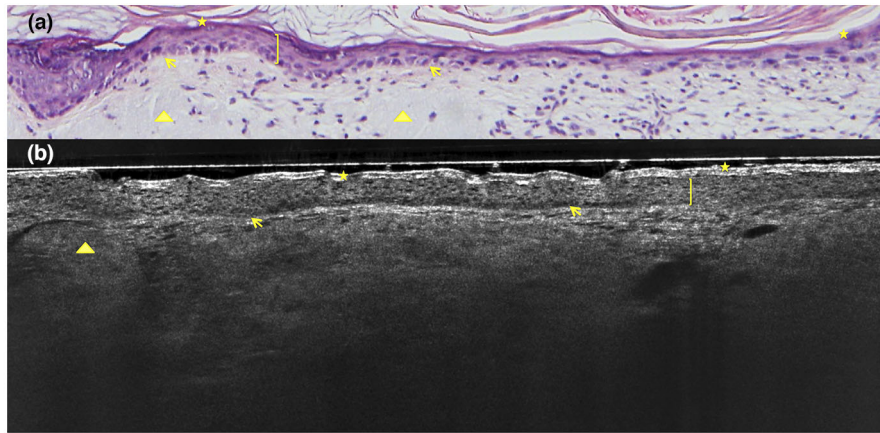


Figure 2 Atrophic AK of the face: H&E stained histology (100 $\times$) (a), LC-OCT in vertical mode (b) Note hyperkeratosis/parakeratosis (star), atypical keratinocytes (bracket) with preserved DEJ and flattened rete ridges (arrow), solar elastosis (triangle). AK, actinic keratosis; DEJ, dermo-epidermal junction; H&E, haematoxylin–eosin; LC-OCT, line-field confocal optical coherence tomography.

sun-damaged skin, AKs and SCCs based on the analysis of cellular, 3D microarchitectural structures and tissue scattering.³⁵

In this article, we focused on LC-OCT features of AK, BD and SCC, in order to correlate the presence of specific descriptors with the stage of KC.

Hyperkeratosis was visible as a hyperreflective multilayer of polygonal cells with sometimes remaining nuclei (parakeratosis). Atypical keratinocytes were polygonal structures with a dark cytoplasm and bright contours, enlarged in comparison with those in healthy epidermis and with inhomogeneous size and shape. The cellular layering was intuitively comparable with the corresponding histological sections. However, it was not always easy to systematically differentiate basal and suprabasal keratinocyte atypia; to this regard, we noticed that the differences were easier detectable with higher grades of dysplasia (BD, SCC). Acantholysis was seen as darker spaces between the epidermal keratinocytes. Also, fragmented (pycnotic) keratinocytes could be detected.

The epidermal thickness systematically and significantly increased with the progression to invasive SCC.

In the dermis, increased vascularization and collagen fibre disarrangement, corresponding to elastosis (collagen reaction), were seen in all types of lesions in both vertical and horizontal sections. Small bright particles, probably corresponding to inflammatory infiltrates, could be visualized in both the epidermal and dermal layers.

We could visualize AKs and their main histopathological correlates with LC-OCT; the device also seemed to be potentially useful in AK subtyping. Although we only analysed a very small sample of AK subtypes (10 hypertrophic, 5 atrophic and 3 bowenoid), we were able to preliminarily observe the characteristic flattening of the rete in hypertrophic AKs, a thinned epidermis

in atrophic AKs and the typical full thickness keratinocyte dysplasia with roundish contours in bowenoid AKs. In early AKs, the dermoscopic structureless red areas interrupted by follicular openings were associated with dilated and tortuous hyporeflexive vessels in the superficial dermis and hyperreflective, follicular hyperkeratosis. A larger, systematic study on AKs subtyping should be performed to further investigate this interesting topic.

In BD, the enlarged atypical keratinocytes built a thickened, acanthotic epidermis with roundish ('bowenoid') contours of the enlarged atypical keratinocytes in all epidermal layers, which we defined as bowenoid pattern. This was the main feature to distinguish BD from AKs and SCCs.

In BDs and AKs, the DEJ was well preserved, seen as a darker band separating the basal keratinocytes from the bright dermal collagen; however, in 7 AKs and 2 BD, some parts of the DEJ were not visible, due to the increased thickness of the stratum corneum, pointing towards the limited penetration depth of LC-OCT, which does not always allow a complete examination of the epidermis and DEJ in thick hyperkeratotic lesions. Possible strategies to partially overcome this limit may be, as in conventional OCT and RCM, focusing on the lesion borders and/or removing thick hyperkeratotic crusts, thus with the risk of bleeding and inflammation impeaching the examination.

Squamous cell carcinomas are invasive, keratinizing tumours, composed of large keratinocytes displaying different grades of atypia, arising from the epidermis and extending into the dermis. In LC-OCT, disarranged masses of large polygonal cells with irregular contours could be seen; roundish bright homogeneous structures corresponding to horn pearls were visible. When visible, the dermis was infiltrated by tumour strands and masses, with increased and irregular vascularization (dilated vessels/neoangiogenesis) and bright collagen bundles

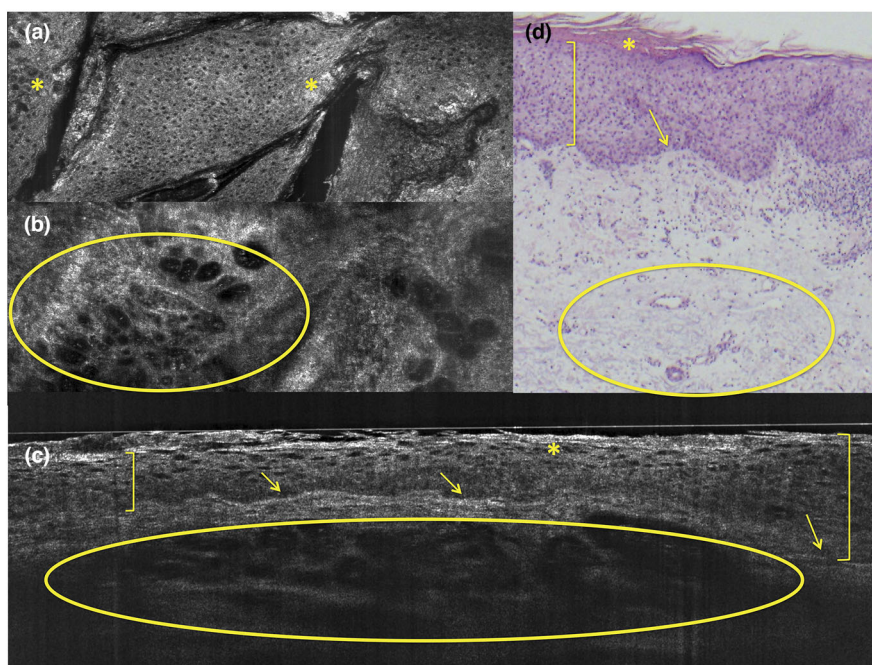


Figure 3 BD of the back: LC-OCT in horizontal mode at the epidermis (a) and junctional (b) level, vertical mode (c) and H&E stained histology (40×) (d). Atypical keratinocytes (bracket), hyperkeratosis with parakeratosis (asterisk), preserved DEJ (arrow), dilated vessels (circle) are seen. BD, Bowen disease; DEJ, dermo-epidermal junction; H&E, haematoxylin–eosin; LC-OCT, line-field confocal optical coherence tomography.

(elastosis/collagen alterations). In the descriptive statistics, these findings were more common in SCCs compared with AKs and BDs, although the model did not reach a statistical significance probably due to the small sample size.

If we take into account RCM, Peppelman and colleagues found architectural disarray in the stratum granulosum–spinosum and nest-like structures in the dermis as predictors of SCC compared to AK, based on 30 lesions.^{29,36} Manfredini *et al.* listed erosion, architectural disarrangement and speckled nucleated cells in the dermis as the most common SCC features; buttonhole vessels were mostly found in in situ SCCs, while irregularly dilated vessels could be detected in invasive SCCs.¹⁷ In horizontal LC-OCT images, which are intuitively comparable to RCM ones, we could describe an atypical honeycombed pattern constituted of dark polygonal cells in different sizes and shapes in all lesions; the grade of atypia increased from AK to SCC. Pyknotic cells were also visible. Collagen alterations/elastosis were seen, but we did not find any predominant vascular morphology.

A supplementary point was to evaluate whether specific LC-OCT descriptors were significantly associated with different stages of keratinocyte cancer. In fact, recognizing the invasiveness of the tumour is necessary for the therapeutic

planning, since most AKs and BDs do not require a surgical approach. We therefore included all predictors that showed a significant odds ratio, in a multivariate multinomial logistic regression with bidirectional elimination based on previous studies about other diagnostic techniques,²⁹ and extrapolated dermoscopic and LC-OCT characteristics that can be useful in the correct classification of different subtypes of KC. In fact, a few patterns immediately help recognize different KC types. For example, a continuous DEJ is a typical feature of AK and BD, but cannot be seen in SCC, where an interrupted DEJ, marked collagen alterations and bright (inflammatory) particles in the dermis are prevalent. Even different types of AKs can be better recognized compared with dermoscopy: flattened rete ridges are typical for hypertrophic AKs, while a thinned epidermis was found in atrophic AKs. The model was conceived to calculate the correct classification rate of LC-OCT for AK, BD and SCC, which was 100%. However, due to overfitting, we based the interpretation of our results and discussion on the univariate multinomial logistic regressions. These results should be considered as preliminary and serve as a hint for future studies on larger samples, including more BD and SCCs compared with AKs.

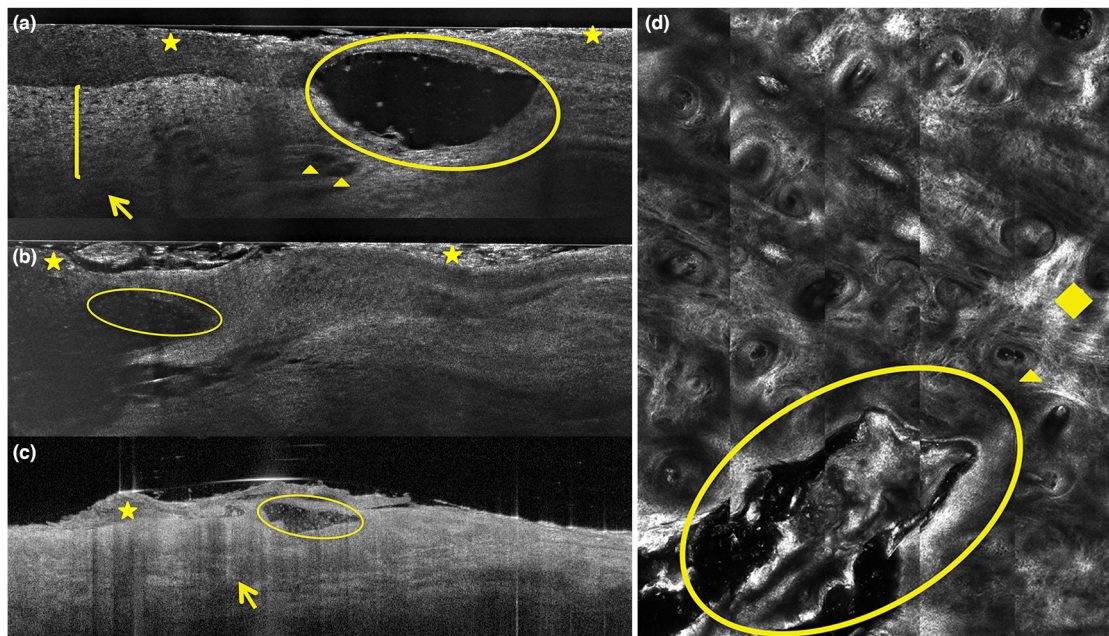


Figure 4 SCC of the scalp in vertical LC-OCT (a, b), OCT (c) and RCM (d). We see the following: a thick hyperkeratotic crust (asterisk), ulceration/blood crust (circle), atypical keratinocytes (bracket) with loss of the DEJ (arrow), dilated vessels (triangle), marked architectural disarrangement and elastosis (rhombus) also at the periphery of the lesion. DEJ, dermo-epidermal junction; H&E, haematoxylin–eosin; LC-OCT, line-field confocal optical coherence tomography; OCT, optical coherence tomography; RCM, reflectance confocal microscopy; SCC, squamous cell carcinoma.

Advantages and limitations of LC-OCT compared with other diagnostic techniques should be briefly discussed. The scanning camera allows the direct navigation of the examined area, as in conventional OCT and RCM 1500®. The unique combination of vertical and horizontal images at nearly cellular resolution makes histopathological correlations easier and reduces diagnostic pitfalls. The limited resolution compared with RCM does not impeach the evaluation of the keratinocyte atypia, but might complicate the differential diagnosis with melanocytic lesions. Offering less penetration depth than conventional OCT, LC-OCT can miss the deeper epidermal layers in the presence of massive hyperkeratosis, so that some thick early lesions might be misevaluated. Only conventional OCT offers a dynamic mode for blood flow.

Conclusions

We believe that the integration of clinical, dermoscopic and imaging data improves the therapeutic planning and follow-up of KC. In our experience, LC-OCT recognizes main features (keratinocyte morphology, architecture and DEJ structure) and allows the diagnosis of different stages of KC with intuitive and reliable histopathological correlations. Our findings open new scenarios in the non-invasive characterization of field cancerization. Further studies are needed to correlate

the LC-OCT images to other histologic AK classifications such as PRO (a model focused on different stages of downward extension of basal keratinocytes ‘protruding’ into the papillary dermis)^{37,38} and to standardize imaging algorithms for AKs, BD and SCCs.

Acknowledgments

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Supporting information

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

Figure S1. Hypertrophic AK of the scalp: in vertical LC-OCT (a) and H&E stained histology (40×): hyperkeratosis/parakeratosis (star) together with atypical keratinocytes (bracket) are seen, while the DEJ is preserved (arrow); solar elastosis is present (triangle).

Figure S2. Detail of the cellular morphology of a clear cell BD in LC-OCT in horizontal mode (a) and H&E stained histology (100×) (b). Hyperkeratosis with parakeratosis (star) and large atypical keratinocytes (bracket) are seen.

Table S1. Descriptive statistics of dermoscopic features of actinic keratosis, Bowen's disease, and squamous cell carci-

noma evaluated in the study with their relative and absolute frequencies.

Table S2. Univariate multinomial logistic regression reporting odds ratios (OR) and *P*-values of main analysed dermoscopic patterns.

Line-field optical coherence tomography: *in vivo* diagnosis of basal cell carcinoma subtypes compared with histopathologyC. Ruini,^{1,2}  S. Schuh,³ C. Gust,¹ B. Kendziora,¹  L. Frommherz,¹  L. E. French,^{1,4}
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Summary

Background. Basal cell carcinoma (BCC) is the most common skin cancer in the general population. Treatments vary from Mohs surgery to topical therapy, depending on the subtype. Dermoscopy, reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) have gained a foothold in daily clinical practice to optimize diagnosis and subtype-oriented treatment. The new technique of line-field confocal OCT (LC-OCT) allows imaging at high resolution and depth, but its use has not yet been investigated in larger studies.**Aim.** To evaluate the main LC-OCT criteria for the diagnosis and subtyping of BCC compared with histopathology, OCT and RCM.**Methods.** In total, 52 histopathologically confirmed BCCs were evaluated for imaging criteria. Their frequency, predictive values and ROC curves were calculated. A multinomial regression with stepwise variables selection to distinguish BCC subtypes was performed.**Results.** Nodular BCCs were mainly characterized by atypical keratinocytes, altered dermoepidermal junction (DEJ), tumour nests in the dermis, dark clefting, prominent vascularization and white hyper-reflective stroma. Superficial BCCs showed a thickening of the epidermis due to a series of tumour lobules with clear connection to the DEJ (string of pearls pattern). Infiltrative BCCs were characterized by elongated hyporeflexive tumour strands, surrounded by bright collagen (shoal of fish pattern). The overall BCC subtype agreement between LC-OCT and conventional histology was 90.4% (95% CI 79.0–96.8).**Conclusion.** LC-OCT allows noninvasive, real-time identification of BCCs and their subtypes in vertical, horizontal and three-dimension mode compared with histology, RCM and OCT. Further larger studies are needed to better explore the clinical applications of this promising device.

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Introduction

Basal cell carcinoma (BCC) is the most commonly occurring type of skin cancer in the general population. Because advanced tumours can be locally destructive and disfiguring, early detection and treatment are essential to limit destructive surgical procedures and their complications, economic burden and patient discomfort.¹ In a real-life setting, clinical and dermoscopic examination helps with identification of

lesions suspicious for BCC. Patients with such lesions may then proceed to diagnostic or therapeutic procedures. Histology is accepted as the gold-standard assessment tool for BCC subtyping, which then directly guides the treatment offered to patients.¹ As biopsies are expensive and time-consuming and carry additional risks, noninvasive diagnostic methods have gained a foothold in daily clinical practice.² Among these, reflectance confocal microscopy (RCM), conventional and high-definition optical coherence tomography (OCT) and multiphoton microscopy have been used to increase diagnostic accuracy and to allow non-invasive BCC subtyping.^{3–9} The main imaging criteria for diagnosing BCCs have been described in numerous studies as having diagnostic sensitivity and specificity values > 90%.^{9–11}

Nevertheless, such technologies have minor disadvantages. Although RCM has a high resolution (horizontal < 1.25 µm, vertical < 5.0 µm), its penetration depth of 200–250 µm only reaches the superficial dermis. By contrast, OCT has a penetration depth of up to 1.5 mm, but its resolution is only about 7.5 µm (lateral) to 5 µm (axial).

The new technique of line-field confocal OCT (LC-OCT) allows the simultaneous display of horizontal and vertical images with both cellular resolution (axial 1.1 µm, lateral 1.3 µm) and a detection depth (~500 µm) that reaches to the mid-dermis. Healthy skin, various skin tumours and mite infestations have all been investigated by LC-OCT in pilot studies.^{1–18} Moreover, a recently published study¹⁸ described the main morphological criteria for BCC subtypes in LC-OCT. The aim of the current work was to evaluate the advantages and limitations of LC-OCT in the diagnosis of BCC and in the differentiation of BCC subtypes validated by histopathology in a clinical setting. A secondary aim was to compare a subgroup of LC-OCT BCC images with corresponding RCM and OCT images of the same lesion, focusing on diagnostic confidence.

Methods

The study protocol was reviewed and approved by the institutional review board of LMU Munich (approval no. 17-699) and written informed consent was obtained from all participants.

Study population

Patients were prospectively recruited and evaluated for the main imaging criteria in the context of a global

study on pigmented and nonpigmented lesions examined with LC-OCT. We later included in the statistical analysis only previously untreated cases that had been completely excised after image review and confirmed as BCCs by subsequent histopathological examination and complete excision: 25 nodular (n)BCCs, 11 superficial (s)BCCs, 5 infiltrative (i)BCCs and 11 nodular–superficial (ns)BCCs. Four of the five iBCCs also included minor nodular components, but were clustered as infiltrative based on their predominant appearance. The nsBCCs had a homogeneous distribution of both components.

Images

Clinical, dermoscopic and LC-OCT images [horizontal, vertical, three-dimensional (3D) mode] were collected in all cases. Additionally and depending on patient consent, OCT images in horizontal (*en face*) view (6 × 6 mm in 31 cases: 13 nBCCs, 9 sBCCs, 2 iBCCs and 7 mBCCs) and RCM multiple Vivastacks (500 × 500 µm) and Vivablocks (5 × 5 mm) images (22 cases: 9 nBCCs, 4 sBCCs, 1 iBCC and 7 nsBCCs). In each case, the whole tumour area was scanned. Images were evaluated by four trained dermatologists (CR, SS, LF, ES) and agreement reached by consensus. Discordant cases were discussed with two dermatologists (DH, JW) who are experts in dermatopathology. Histopathological slides were analysed by the senior pathologist of each centre.

Imaging devices

The LC-OCT system (DAMAE Medical, Paris, France) is classified as a class 1 supercontinuum laser, and uses a central wavelength of 800 nm. Combining the principle of OCT interferometry with the spatial filtering of RCM, the device collects multiple A-scans parallel to the skin surface to a depth of ~500 µm, while constantly adjusting its focus. It has three imaging modalities displayed as a grey scale: vertical or *en coupe*, horizontal or *en face* and 3D stack for a 3D reconstruction, with a vertical and horizontal field of view of 1.2 × 0.5 mm². Details have been described in previous studies.^{14,17,19–21} Conventional OCT images were acquired with Vivosight (Michelson Diagnostics Ltd, Maidstone, Kent, UK), RCM images with Vivascope 1500 (Mavig GmbH, Munich, Germany) and clinical–dermoscopic images with Fotofinder (FotoFinder GmbH, Berlin, Germany) and Dermogenius 2 (Dermoscan GmbH, Regensburg, Germany).

Imaging features

The main diagnostic patterns were selected based on previous publications. The dermoscopic features are described in Table 1. For LC-OCT, a selection of criteria based on histological diagnostic features and previously described OCT and RCM terminology was developed (Table 1).^{22–25} In addition, image quality and confidence with diagnosis and subtype were reported semiquantitatively (low < 50%, average 50–75%, high > 75%).

Statistical analysis

For descriptive statistics, mean $\pm$ SD were calculated for numerical variables, while absolute numbers with percentage values were used for nominal variables. To evaluate the diagnostic accuracy of LC-OCT in detecting different BCC subtypes, the sensitivity, specificity, positive predictive value (PPV), negative predictive

value (NPV), and area under the curve of the receiver-operating characteristic curve were calculated. Dermatopathology was considered the gold standard. Multinomial regression with stepwise selection of variables was used to search for LC-OCT characteristics that would differentiate between BCC subtypes. Variables without explanatory value as measured by the Akaike information criterion were excluded by bidirectional elimination. All statistics were performed in R software (V3.6.0, 2; R Foundation for Statistical Computing, Vienna, Austria). $P < 0.05$ was considered statistically significant.

Results

Epidemiology

In total, 52 patients (35 men, 17 women, mean age 71 years) with Fitzpatrick skin phototypes I–III with

Table 1 Main line-field confocal optical coherence tomography in detecting different basal cell carcinoma subtypes evaluated in the study with their relative and absolute frequencies.

	BCC subtype									
	All		Nodular		Superficial		Infiltrative		Nodular–superficial	
	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N
Orientation										
<i>En coupe</i> (vertical)										
Epidermis										
Hyperkeratosis	32.7	17/52	32.0	8/25	9.1	1/11	60.0	3/5	45.5	5/11
Thinning	46.2	24/52	44.0	11/25	36.4	4/11	40.0	2/5	63.6	7/11
Scales	26.9	14/52	24.0	6/25	27.3	3/11	40.0	2/5	27.3	3/11
Keratin plugs	5.8	3/52	8.0	2/25	0	0/11	0	0/5	9.1	1/11
Ulceration	21.2	11/52	16.0	4/25	18.2	2/11	20.0	1/5	36.4	4/11
Atypical keratinocytes	76.9	40/52	68.0	17/25	81.8	9/11	0	0/5	100.0	11/11
DEJ and dermis										
Alteration of the DEJ profile	98.1	51/52	96.0	24/25	100.0	11/11	100.0	5/5	100.0	11/11
Tumour nests/lobules	98.1	51/52	100.0	25/25	100.0	11/11	80.0	4/5	100.0	11/11
Clefting	96.2	50/52	100.0	25/25	90.9	10/11	80.0	4/5	100.0	11/11
Prominent vessels/neoangiogenesis	96.2	50/52	92.0	23/25	100.0	11/11	100.0	5/5	100.0	11/11
Bright structures	42.3	22/52	56.0	14/25	18.2	2/11	20.0	1/5	45.5	5/11
Shoal of fish pattern	13.5	7/52	4.0	1/25	0	0/11	100.0	5/5	9.1	1/11
String of pearls pattern	42.3	22/52	12.0	3/25	90.9	10/11	20.0	1/5	72.7	8/11
White hyper-reflective stroma	94.2	49/52	92.0	23/25	90.9	10/11	100.0	5/5	100.0	11/11
Black areas/cysts	19.2	10/52	12.0	3/25	18.2	2/11	40.0	2/5	27.3	3/11
Cell polarization	67.3	35/52	64.0	16/25	72.7	8/11	80.0	4/5	63.6	7/11
<i>En face</i> (horizontal)										
Atypical honeycomb	76.9	40/52	68.0	17/25	81.8	9/11	0	0/5	100.0	11/11
Clefting	53.8	28/52	60.0	15/25	45.5	5/11	40.0	2/5	54.5	6/11
Cord-like structures	11.5	6/52	8.0	2/25	36.4	4/11	0	0/5	0	0/11
Dark silhouettes	46.2	24/52	40.0	10/25	63.6	7/11	60.0	3/5	36.4	4/11
Tumour nests/lobules	65.5	33/52	72.0	18/25	36.4	4/11	60.0	3/5	72.7	8/11
Canalicular vessels	71.2	37/52	76.0	19/25	72.7	8/11	80.0	4/5	54.5	6/11
Collagen alterations	98.1	51/52	100.0	25/25	90.9	10/11	100.0	5/5	100.0	11/11

BCC, basal cell carcinoma; DEJ, dermoepidermal junction.

histologically confirmed BCCs were enrolled in the study. Most BCCs arose in the head and neck area (51.9%), followed by the trunk (34.6%) and limbs (13.5%).

Diagnostic confidence

Diagnostic confidence for BCC subtype (high, average and low, respectively) was 44.2%, 42.3% and 13.5% for dermoscopy, and 78.8%, 15.4% and 5.8% for LC-OCT. LC-OCT increased the examiners' diagnostic confidence by 36.5%. Diagnostic confidence (high and average, respectively) was 68% and 24% for OCT, and 44% and 31.5% for RCM was: high. LC-OCT image quality was high in 75% of cases and average in the remaining 25%.

Imaging features

The main dermoscopic features are shown in Table S1, and the main overall LC-OCT features of BCCs are shown in Table 1.

In vertical mode, nBCCs were mainly characterized by atypical keratinocytes in the epidermis above them (68%), an altered dermoepidermal junction (DEJ) profile as determined by connected or underlying tumour lobules (96%), tumour nests/lobules in the dermis (100%), dark clefting (100%), prominent vessels (92%) and white hyper-reflective stroma (92%). In horizontal mode, an atypical honeycomb pattern (68%), tumour nests/lobules (72%), dark silhouettes (40%) and collagen alterations (100%) were seen (Figs 1 and 2; Fig. S1, Video S1, Table 1).

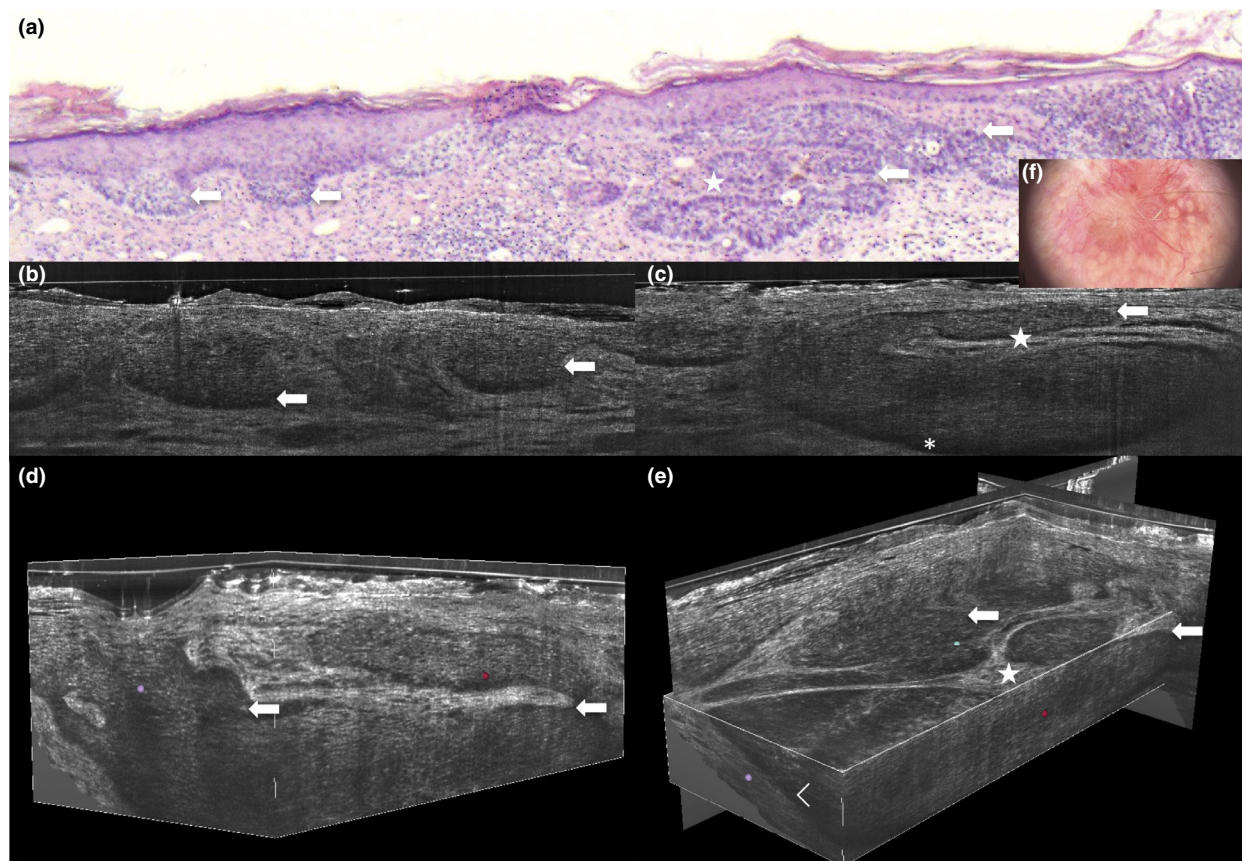


Figure 1 (a–f) Appearance of a nodular-superficial basal cell carcinoma of the face in a 60-year-old man as visualized by (a) conventional microscopy (haematoxylin and eosin, original magnification $\times 100$), (b–e) line-field confocal optical coherence tomography in (b, c) vertical and (d, e) three-dimensional mode, and (f) dermoscopy (original magnification $\times 10$). Note the hyporeflexive ovoid structures arranged in a string of pearls pattern (arrow), together with the beginning of nodular components (arrow), which have lost their connection to the epidermis, separated by a discrete stromal reaction (star).

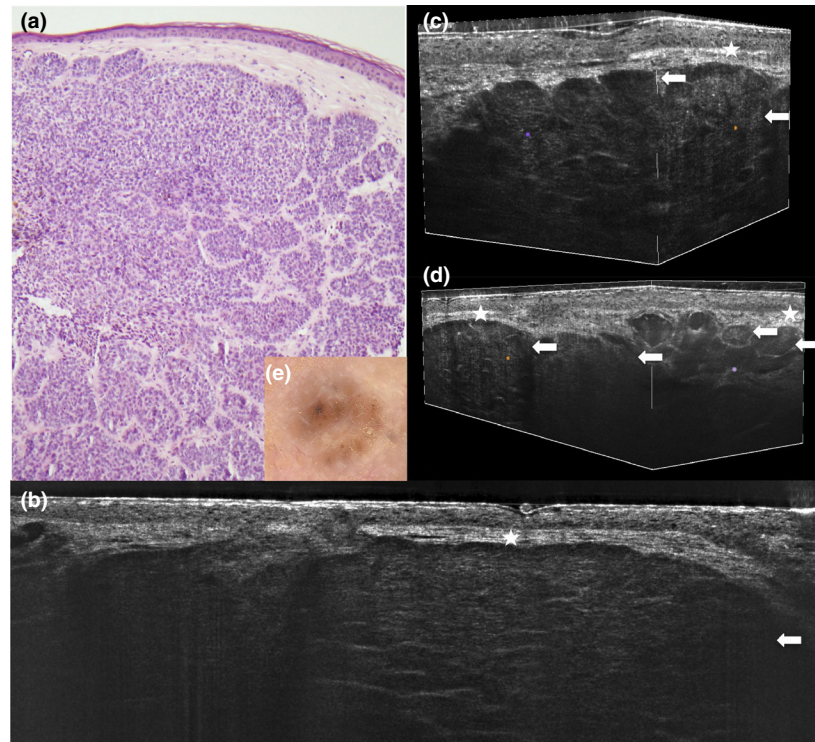


Figure 2 (a–e) Appearance of a nodular–micronodular basal cell carcinoma of the face in a 75-year-old woman, as visualized by (a) conventional microscopy (haematoxylin and eosin, original magnification $\times 140$); (b–d) line-field confocal optical coherence tomography in (b) vertical and (c, d) three-dimensional mode, and (e) dermoscopy (original magnification $\times 10$). Note the hyporeflective ovoid structures/lobules of different sizes in the dermis (arrow), pushing towards the thinned epidermis and surrounded by stromal reaction (star).

For sBCCs, atypical keratinocytes were noticed in 81.1% of cases, while an altered DEJ profile, tumour nests/lobules, white hyper-reflective stroma and prominent vessels were seen in 100% of cases. Cell polarization was seen in 72.7% of cases, and the ‘string of pearls’ pattern in 90.9% (Figs 1 and 3, Figs S1 and S3).

By contrast, the infiltrative subtypes were characterized by the so-called ‘shoal of fish’ pattern (100%) (Fig. 4). Mixed subtypes equally displayed the ‘string of pearls’ patterns as well as deeper ovoid tumour nests/lobules with no connection to the epidermis.

Agreement

The overall BCC subtype agreement between LC-OCT and conventional histology was 90.4% (95% CI 79.0–96.8), compared with 84% for OCT and 62.5% for RCM.

The sensitivity, specificity, PPV and NPV values of LC-OCT for different BCC subtypes are shown in Table 2, with ROC curves in Fig. 5. Multinomial

regression with stepwise selection of variables identified the following features as most useful in distinguishing BCC subtypes: epidermal thinning, atypical honeycomb pattern, prominent vessels/neoangiogenesis, shoal of fish pattern, string of pearls pattern and white hyper-reflective stroma. Bidirectional elimination excluded (*en face*) tumour nests and (*en face*) clefting (Tables S2 and S3).

Discussion

In vivo BCC morphology has been widely characterized using, among others, the techniques of RCM and OCT. Longo *et al.* developed an RCM algorithm for differentiating BCC subtypes, with the key criteria being cord-like structures for sBCCs, large tumour nests and clefting for nBCCs, and dark silhouettes and abundant bright compact collagen for iBCCs.¹⁰ Conventional OCT has been used for the noninvasive characterization of BCCs,^{2,26–28} and the specific features identified were hyporeflective ovoid structures originating from the stratum basale/DEJ,

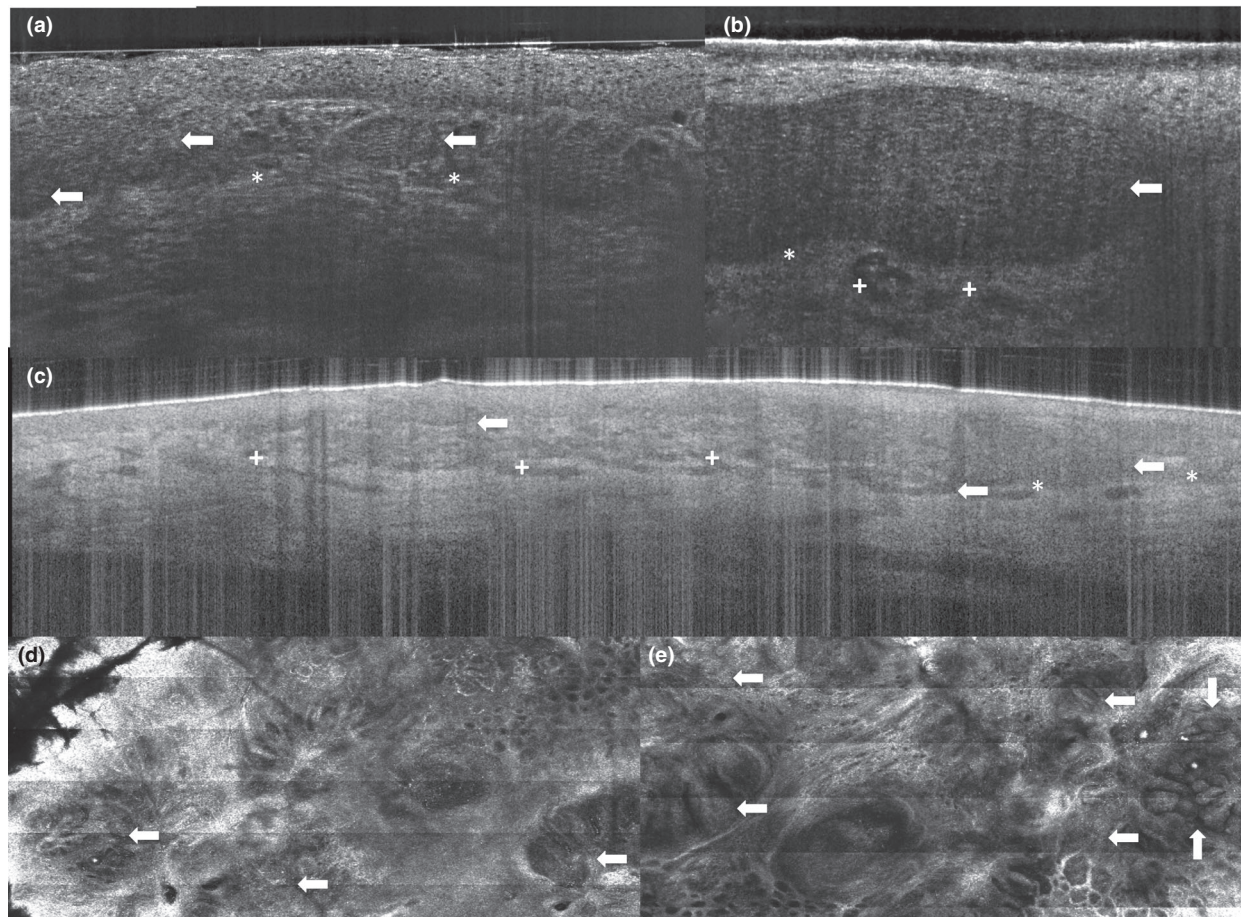


Figure 3 (a–e) Superficial basal cell carcinoma of the leg in a 54-year-old woman as visualized by (a, b) line-field confocal optical coherence tomography in vertical mode, (c) optical coherence tomography and (d,e) Vivastack reflectance confocal microscopy. Note the hyporeflective ovoid structures or tumour nests/lobules of different sizes in the dermis arranged in a string of pearls pattern (arrow), surrounded by stromal reaction (star) and dilated vessels (+).

corresponding to tumour nests, either with contact to the DEJ (string of pearls pattern) in sBCCs, or separated from the DEJ in nBCCs; and a dark rim, white hyper-reflective stroma, cysts and shoal of fish structures in iBCCs.¹¹ Dedicated Cochrane reviews have confirmed a role for both RCM and OCT in the diagnosis of clinically challenging BCCs.^{9,12} In a pilot study, a combination of OCT and RCM showed good correlation to key histopathological features of infiltrative BCCs.²⁹

LC-OCT can be seen as a method for combining the aforementioned diagnostic tools. LC-OCT images can be intuitively evaluated by physicians trained in non-invasive diagnostic technologies and with at least basic knowledge of skin histopathology. In fact, vertical (*en coupe*) scans are directly comparable to OCT images and histology, while horizontal (*en face*) scans

can be related to RCM and dermoscopy. Preliminary studies conducted with available LC-OCT prototypes reported good correlations with histopathology in pilot settings.^{14,30} However, there is a lack of systematic studies on large numbers of cases, with only one very recent study analysing 66 BCCs of pure histological subtypes.¹⁸ Suppa *et al.* described lobules, blood vessels and small bright cells within epidermis as the most common criteria for BCCs. They also associated hemispheric lobules, connection with the epidermis and absence of stretching of the stroma with sBCCs; macrolobules, absence of connection to the epidermis with nBCCs; and branched lobules with iBCCs.¹⁸ We observed similar features, but we have described our nomenclature based on the standard histological patterns, known RCM criteria for cytology and known OCT criteria for morphology.

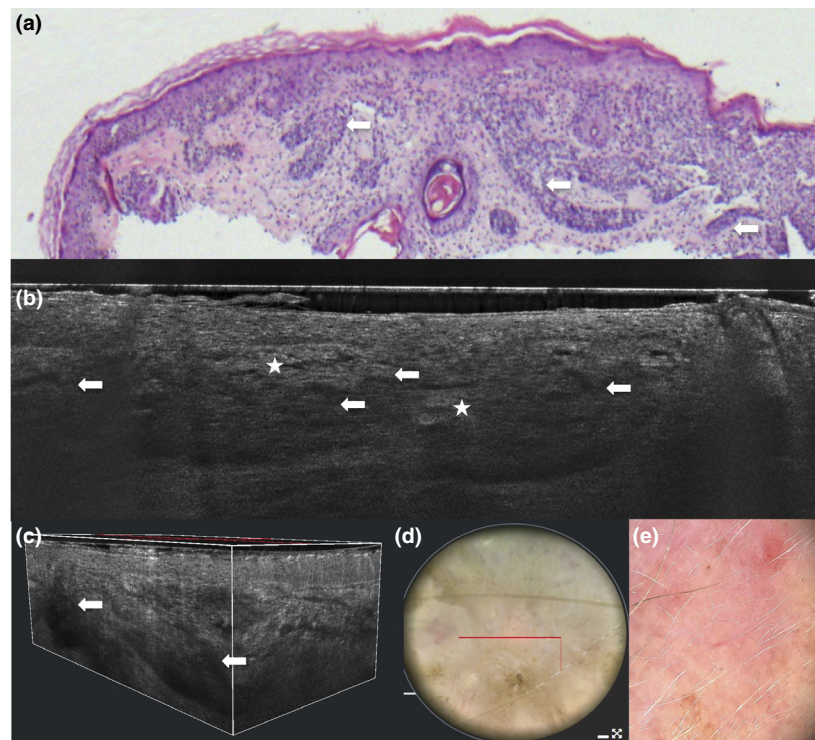


Figure 4 (a–e) Infiltrative basal cell carcinoma of the face in a 60-year-old man as visualized by (a) conventional microscopy (haematoxylin and eosin, original magnification $\times 40$), (b, c) line-field confocal optical coherence tomography in (b) vertical and (c) three-dimensional mode, (d) clinical examination and (e) dermoscopy (original magnification $\times 10$). Note the bizarrely configured, hyporeflexive, branched strands arranged in a shoal of fish pattern (arrow), separated by a discrete stromal reaction (star).

The most intuitive BCC feature in our analysis is the tumour nest/lobule, which corresponds to its histological counterpart. Dark peritumoral clefting corresponding to mucin clearly delimitates the nests, which are surrounded by a bright collagenic stromal reaction in most cases.

Compared with conventional OCT, LC-OCT provides a higher resolution, which allows visualization of cellular components. In particular, larger cells, such as keratinocytes and activated melanocytes, can be clearly seen. In BCCs, slightly atypical keratinocytes in the epidermis and atypical cells in the tumour/lobules (described as cells of different sizes, shapes and contours) are visible. The cells in the tumour nests/lobules are hyporeflexive with a hyper-reflective border and can either be polarized in overlapping strands or sometimes occur in a classic peripheral palisading. Analogously, pigmented BCCs sometimes show more hyper-reflective components, possibly corresponding to melanocytes infiltrating the BCC, or to melanophages or pigmented keratinocytes, in line with previous experience with RCM and OCT.

The alteration of the DEJ seen in RCM and OCT becomes an alteration of the DEJ profile in LC-OCT compared with healthy skin, as tumour nests/lobules appear to be either connected to the epidermis (in sBCCs) or pushing it upwards (in nBCCs).

In our experience, nBCCs are extremely well characterized with ovoid nests/lobules in the dermis, pushing against the DEJ and causing a thinning of the epidermal layers. Micronodular tumours were also distinguishable by their smaller lobular components. Sometimes, dark holes were seen inside the nests, probably due to cysts and/or necrosis.

In the case of sBCCs, the tumour nests/lobules, which were slightly elongated, were clearly identifiable as a series of small ovoid nests/lobules connected with the DEJ and with each other through streamlined cords (string of pearls pattern). In contrast to conventional OCT, however, the epidermis does not appear irregularly thickened, but can be mostly distinguished from the tumour lobules connected to it.

Infiltrative tumours are usually more difficult to diagnose, as they do not have well-defined roundish

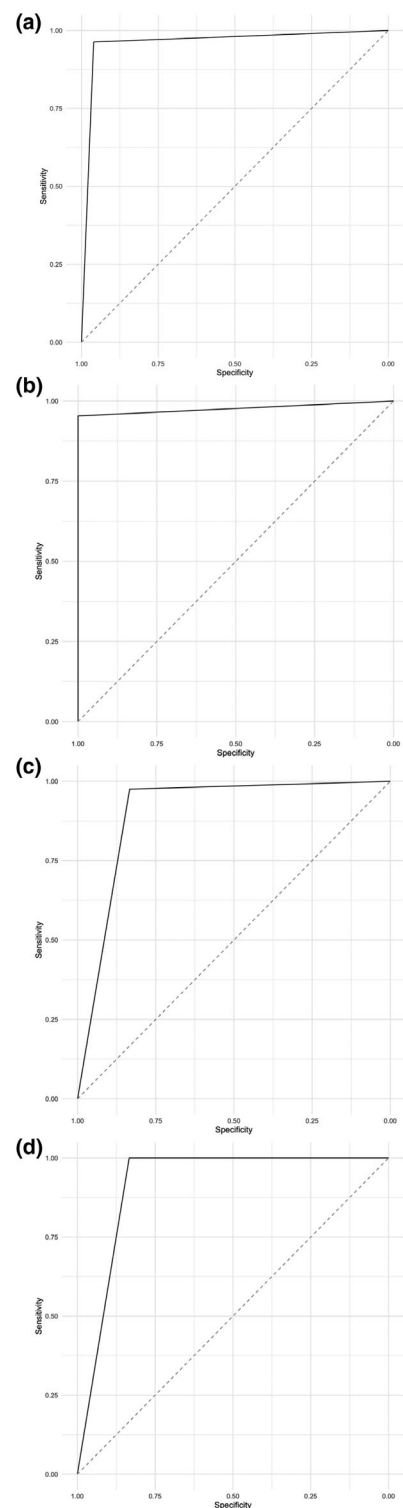
Table 2 Diagnostic accuracy of line-field confocal optical coherence tomography in detecting different basal cell carcinoma subtypes.

BCC type	Point estimates (95% CI)
Nodular	
Apparent prevalence	0.48 (0.34–0.62)
True prevalence	0.48 (0.34–0.62)
Sensitivity	0.96 (0.80–1.00)
Specificity	0.96 (0.81–1.00)
Positive predictive value	0.96 (0.80–1.00)
Negative predictive value	0.96 (0.81–1.00)
Positive likelihood ratio	25.92 (3.78–177.68)
Negative likelihood ratio	0.04 (0.01–0.28)
Kappa	0.92 (0.82–1.00)
AUC	0.96 (0.91–1.00)
Superficial	
Apparent prevalence	0.17 (0.08–0.30)
True prevalence	0.21 (0.11–0.35)
Sensitivity	0.82 (0.48–0.98)
Specificity	1.00 (0.91–1.00)
Positive predictive value	1.00 (0.66–1.00)
Negative predictive value	0.95 (0.84–0.99)
Positive likelihood ratio	Inf (NaN–Inf)
Negative likelihood ratio	0.18 (0.05–0.64)
Kappa	0.88 (0.71–1.00)
AUC	0.98 (0.95–1.00)
Infiltrative BCCs	
Apparent prevalence	0.12 (0.04–0.23)
True prevalence	0.10 (0.03–0.21)
Sensitivity	1.00 (0.48–1.00)
Specificity	0.98 (0.89–1.00)
Positive predictive value	0.83 (0.36–1.00)
Negative predictive value	1.00 (0.92–1.00)
Positive likelihood ratio	47.00 (6.76–326.73)
Negative likelihood ratio	0.00 (0.00–NaN)
Kappa	0.90 (0.70–1.00)
AUC	0.92 (0.75–1.00)
Nodular–superficial BCCs	
Apparent prevalence	0.23 (0.13–0.37)
True prevalence	0.21 (0.11–0.35)
Sensitivity	0.91 (0.59–1.00)
Specificity	0.95 (0.83–0.99)
Positive predictive value	0.83 (0.52–0.98)
Negative predictive value	0.97 (0.87–1.00)
Positive likelihood ratio	18.64 (4.76–72.94)
Negative likelihood ratio	0.10 (0.01–0.62)
Kappa	0.83 (0.65–1.00)
AUC	0.90 (0.79–1.00)

AUC, area under the curve; Inf, infimum; NaN, not a number (undefined).

nests but rather elongated, slightly hyporeflective strands/branched lobules, surrounded by a bright collagen reaction. This feature defines the ‘shoal of fish’ pattern, also visible in OCT.

BCC subtyping is crucial for choosing an appropriate therapy, while superficial and thin nodular BCCs can

**Figure 5** Receiver-operating characteristic curves: (a) nodular basal cell carcinoma (BCC), (b) superficial BCC, (c) infiltrative BCC and (d) nodular–superficial BCC.

be treated with local therapy options, thick nodular and infiltrative BCCs should be excised with (Mohs) surgery. Up to 40% of BCCs in daily clinical practice are of mixed subtype, and a normal punch biopsy is often too small to ensure complete lesion sampling.³¹

In vivo mapping with LC-OCT is able to scan the whole lesion and is able to identify the individual components of mixed tumours, providing an advantage compared with standard punch biopsies.

In our analysis, one case of superficial BCC was wrongly evaluated as nodular–superficial, probably due to numerous sebaceous glands acting as confounders; such entities appear as roundish hyporeflective structures with hyper-reflective borders, usually containing lobules of large (sebaceous) cells. To avoid confounding, we suggest combined evaluation of vertical and horizontal images, as sebaceous glands and sebaceous hyperplasia appear in horizontal mode as sharply demarcated, concentric, roundish structures in continuity with hair follicles, in contrast to BCC tumour nests.

In LC-OCT horizontal sections, the following BCC patterns were found: an atypical honeycomb pattern defined by polygonal bright keratinocytes of different sizes, shapes and contours; canalicular blood vessels; bright collagen stromal reaction/elastosis; and ovoid hyporeflective tumour nests with dark clefting and palisading. In our experience, these characteristics appeared less well defined than with RCM.

In our opinion, the main advantages of LC-OCT are the nearly cellular resolution and the good penetration depth reaching the dermis, combined with the instant switch from vertical to horizontal mode, the user-friendly software and the fast camera-guided image acquisition in three dimensions, which allows navigation of the whole tumour.

Nevertheless, some limitations exist. For example, distinguishing between nBCCs and sBCCs can sometimes be difficult, the tight connection of the tumour islands to the DEJ might be overlooked. In addition, some deep nodular components might not be visualized because of limits to the penetration depth of LC-OCT (which is less than conventional OCT), so that the possibility of missing deeper nodular components exists. For the same reason, determination of BCC thickness of the BCC is limited with LC-OCT compared with conventional OCT, as it is with RCM. Infiltrative BCCs require caution, as elongated tumour strands can sometimes be misinterpreted as blood vessels by nonexpert observers. Some significant differential diagnoses, for example with melanocytic tumours, are more difficult using LC-OCT compared with RCM because of the slightly lower resolution of the LC-OCT

device. All the cited technologies can encounter limits in scanning difficult-to-reach anatomical areas, such as the inner eyelids. In doubtful cases, short-term follow-up or a biopsy should be performed.

This work was limited by the smaller number of iBCCs and RCM/OCT acquisitions. Moreover, the multiple but small Vivastacks images for mapping the whole tumour area might be responsible for the lower diagnostic confidence attributed to RCM. A larger, systematic comparison study should be conducted to further analyse the advantages and pitfalls of the different technologies.

Conclusion

Our study describes the most common LC-OCT features of BCCs compared with histological findings, and shows that the device provides significant additional morphological details compared with naked eye examination and dermoscopy for diagnosing BCCs and their histological subtypes. This could have important practical consequences as it allows the clinician to immediately assign the correct treatment for the patient. Similar to other, already established, noninvasive diagnostic methods, LC-OCT is quick, painless and intuitively comparable to histology following dedicated training.

Acknowledgement

We thank DAMAE Medical for providing the LC-OCT device used for this study.

What's already known about this topic?

- The novel imaging technique of LC-OCT has been shown in small case series to be able to noninvasively characterize healthy skin and potentially nonmelanoma skin cancer, as a result of its good resolution and penetration depth.
- There is to date just one systematic study defining the LC-OCT diagnostic criteria for BCC.

What does this study add?

- LC-OCT is useful for the *in vivo* diagnosis and characterization of BCC subtypes with high sensitivity and specificity compared with histology.
- The comparison with other imaging technologies shows the advantages and limitations of this new imaging method.

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Supporting Information

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

Figure S1. (a–d) Nodular basal cell carcinoma on the face of a 75-year-old woman as visualized under (a,b) line-field confocal optical coherence tomography in (a) vertical and (b) horizontal mode, (c) optical coherence tomography and (d) Vivastack reflectance confocal microscopy. Note the hyporeflexive ovoid structures/lobules of different size in the dermis (arrow), surrounded by stromal reaction (*).

Figure S2. (a–d) Superficial basal cell carcinoma on the back of a 47-year-old man as visualized under (a) conventional microscopy (haematoxylin and eosin, original magnification $\times 100$), (b) line-field confocal optical coherence tomography in vertical mode and (c) dermoscopy (original magnification $\times 10$). Note the hyporeflexive ovoid structures arranged in a string of pearls pattern (arrow), surrounded by a dark rim corresponding to peripheral clefting (*).

Figure S3. (a–c) Superficial basal cell carcinoma of the leg in a 54-year-old woman as visualized under (a) conventional microscopy (haematoxylin and eosin, original magnification $\times 100$), (b) line-field confocal optical coherence tomography in vertical mode and (c) dermoscopy (original magnification $\times 10$). Note the hyporeflexive ovoid structures arranged in a string of pearls pattern (arrow), surrounded by dark rims corresponding to peripheral clefting (*). A hyperkeratotic crust (triangle) overlies the epidermal layer. Additionally, a sebaceous gland (rhombus) is visible.

Table S1. Main dermoscopy features of BCCs evaluated in the study with their relative and absolute frequencies.

Table S2. Multinomial logistic regression with stepwise selection of variables to search for LCOCT characteristics helping in the distinction of BCC subtypes.

Table S3. Final model.

Video S1. Line-field confocal optical coherence tomography three-dimensional acquisition of a pigmented nodular basal cell carcinoma.

Article

Line-Field Confocal Optical Coherence Tomography Increases the Diagnostic Accuracy and Confidence for Basal Cell Carcinoma in Equivocal Lesions: A Prospective Study

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Simple Summary: Basal cell carcinoma is the most frequently occurring type of skin cancer. Its treatment can be either local or surgical depending on its subtype and extension, with early recognized and superficial cases being easier to treat. Some of them, however, display unspecific features, making diagnosis difficult. Non-invasive devices such as line-field confocal optical coherence tomography (LC-OCT) are able to recognize morphological features of different BCC subtypes with a good correlation to histopathology. We decided to study their application to clinically doubtful BCC cases.

Abstract: Diagnosing clinically unclear basal cell carcinomas (BCCs) can be challenging. Line-field confocal optical coherence tomography (LC-OCT) is able to display morphological features of BCC subtypes with good histological correlation. The aim of this study was to investigate the accuracy of LC-OCT in diagnosing clinically unsure cases of BCC compared to dermoscopy alone and in distinguishing between superficial BCCs and other BCC subtypes. Moreover, we addressed pitfalls in false positive cases. We prospectively enrolled 182 lesions of 154 patients, referred to our department to confirm or to rule out the diagnosis of BCC. Dermoscopy and LC-OCT images were evaluated by two experts independently. Image quality, LC-OCT patterns and criteria, diagnosis, BCC subtype, and diagnostic confidence were assessed. Sensitivity and specificity of additional LC-OCT were compared to dermoscopy alone for identifying BCC in clinically unclear lesions. In addition, key LC-OCT features to distinguish between BCCs and non-BCCs and to differentiate superficial BCCs from other BCC subtypes were determined by linear regressions. Diagnostic confidence was rated as “high” in only 48% of the lesions with dermoscopy alone compared to 70% with LC-OCT. LC-OCT showed a high sensitivity (98%) and specificity (80%) compared to histology, and these were even higher (100% sensitivity and 97% specificity) in the subgroup of lesions with high diagnostic confidence. Interobserver agreement was nearly perfect (95%). The combination of dermoscopy and LC-OCT reached a sensitivity of 100% and specificity of 81.2% in all cases and increased to sensitivity of 100% and specificity of 94.9% in cases with a high diagnostic confidence. The performance of LC-OCT was influenced by the image quality but not by the anatomical location of the lesion. The most specific morphological LC-OCT criteria in BCCs compared to non-BCCs were: less defined dermoepidermal junction (DEJ), hyporeflexive tumor lobules, and dark rim. The most relevant features of the subgroup of superficial BCCs (sBCCs) were: string of pearls pattern and absence of epidermal thinning. Our diagnostic confidence, sensitivity, and specificity in detecting BCCs in the context of clinically equivocal lesions significantly improved using LC-OCT in comparison to dermoscopy only. Operator training for image acquisition is fundamental to achieve the best results.

Not only the differential diagnosis of BCC, but also BCC subtyping can be performed at bedside with LC-OCT.

Keywords: basal cell carcinoma; dermoscopy; line-field confocal optical coherence tomography; bedside histology; skin imaging; non-invasive diagnostics in dermatology

1. Introduction

In recent decades, basal cell carcinoma (BCC) has progressed towards being the most frequently occurring type of skin cancer [1,2]. The diagnostic gold standard includes a biopsy or excision of the suspicious lesion and histopathological examination, resulting in a cost- and time-intensive process [3,4]. Treatment depends mainly on the histopathological subtype, with deep and nodular or infiltrating BCCs requiring a complete surgical excision, while superficial tumors can benefit from cryotherapy, lasers, or topical drugs such as imiquimod [2,3]. Therefore, the bedside diagnosis and subtyping of BCC are crucial for treatment planning. Clinical and dermoscopic examinations are commonly used in the daily clinical practice to diagnose BCCs, but non-invasive optical diagnostic methods, such as optical coherence tomography (OCT) and reflectance confocal microscopy (RCM), have shown a high potential in early detection of clinically unclear BCCs, with diagnostic sensitivity and specificity over 90% [5–7]. Additionally, the new line-field confocal OCT (LC-OCT), with higher penetration depth than RCM (500 μm compared to 250 μm) and higher resolution than OCT (1 μm compared to 7.5–10 μm) [8,9], has recently been used to detect BCCs non-invasively according to morphological criteria in preliminary studies [10–12]. However, there are no studies focused on clinically unclear lesions.

The aims of this study were: to verify in a real-life setting the diagnostic value of LC-OCT for clinically unclear BCC cases and to test for the ability to distinguish between superficial and other BCC subtypes, compared to dermoscopy and histopathology. Moreover, we reviewed morphological LC-OCT criteria for BCCs and provide an overview of the main diagnostic pitfalls.

2. Materials and Methods

This prospective study was performed at the Departments of Dermatology of the University of Munich and the University of Augsburg in Germany between November 2019 and February 2021. We prospectively recruited 182 clinically unclear lesions of 154 patients that were referred to our imaging departments from the general outpatient and inpatient departments and from external specialists in order to confirm or to rule out BCC prior to biopsy or excision. Clinical, dermoscopic (FotoFinder GmbH, Germany and Dermogenius-Dermoscan GmbH, Regensburg, Germany), and LC-OCT images of the cases were acquired and analyzed by two blinded imaging experts (CR, CG). Discordant cases were reviewed by a third expert (ES, SS, or JW). After the non-invasive imaging, surgical excision followed by histopathological examination was performed in all lesions.

For every case, the patient's age, sex, and the anatomical site of the lesion were registered. Afterwards, dermoscopy was performed and a dermoscopic diagnosis was registered together with a confidence level ranging from 1 = high (>75% confidence) to 2 = intermediate (>50% confidence) to 3 = low (<50% confidence). Finally, the lesions were scanned with the LC-OCT device (DAMAE Medical, Paris), which uses a class 1 supercontinuum laser with a central wavelength of 800 nm to create various A-scans up to a depth of approximately 500 μm . Three imaging modalities are available: vertical or en-coupe, horizontal or en-face, and a 3D reconstruction either in a vertical or horizontal field of view. Details are described elsewhere [8,9].

The following features were registered: LC-OCT diagnosis, confidence, image quality, and whether LC-OCT could rule out a BCC diagnosis or not. LC-OCT image quality was scored from 1 (high: perfect image, no artefacts) to 2 (low: lower resolution, minor artefacts)

to 3 (insufficient: major artefacts, unreadable). Confidence level was measured as described above for dermoscopy.

We calculated the frequency of the main descriptive morphological parameters used for the identification of BCCs in LC-OCT and their frequency as previously described in published studies [11] (Table 1). Key features useful for differentiating BCCs from non-BCCs and to differentiate between superficial BCC and other subtypes were identified by logistic regression.

To assess the LC-OCT performance, diagnostic accuracy, sensitivity, and specificity for BCCs compared to dermoscopy and to the gold standard histology were calculated using McNemar's tests. Calculation was performed for all lesions and additionally in the subgroup of cases with a confidence level rated as "high" by at least one of the observers.

The study was approved by the ethical committee of the LMU Munich (Protocol Number 17-699).

Table 1. LC-OCT parameters.

(a) Epidermal LC-OCT parameters vertical								
EPIDERMAL LC-OCT PARAMETERS VERTICAL	Parameters used in logistic regression for:		Not-BCC (n = 69)	All BCC (n = 113)	Superficial BCC (n = 35)	Nodular BCC (n = 52)	Infiltrative BCC (n = 4)	Mixed BCC (n = 21)
	BCC vs. non BCC	Superficial BCC vs. other pure BCC subtypes (nodular, fibrosing)						
Hyperkeratoses	x		40 (58.0)	38 (33.6)	13 (37.1)	20 (38.5)	1 (25.0)	4 (19.0)
Thinning of the epidermis	x	x	5 (7.2)	48 (42.5)	7 (20.0)	34 (65.4)	1 (25.0)	6 (28.6)
Bowenoid morphology			14 (20.3)	5 (4.4)	3 (8.6)	2 (3.8)	0 (0)	0 (0)
Acanthosis, verrucous surface			11 (15.9)	4 (3.5)	1 (2.9)	2 (3.8)	0 (0)	1 (4.8)
Scales			39 (56.5)	34 (30.1)	12 (34.3)	14 (26.9)	2 (50.0)	6 (28.6)
Keratin plugs/horn cysts			17 (24.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ulceration			15 (21.7)	35 (31)	9 (25.7)	17 (32.7)	2 (50.0)	7 (33.3)
Pagetoid cells			3 (4.3)	1 (0.9)	0 (0)	1 (1.9)	0 (0)	0 (0)
Ovoid concentric structures (sebaceous gland)			19 (27.5)	7 (6.2)	0 (0)	4 (7.7)	1 (25.0)	2 (9.5)
Atypical junctional nests			2 (2.9)	1 (0.9)	0 (0)	1 (1.9)	0 (0)	0 (0)
Tumour strands			6 (8.7)	2 (1.8)	0 (0)	2 (3.8)	0 (0)	0 (0)
Wave pattern/junctional nests			5 (7.2)	1 (0.9)	0 (0)	0 (0)	1 (25.0)	0 (0)
Dermal/deep nests			4 (5.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Atypical honeycombed pattern			49 (71.0)	74 (65.5)	19 (54.3)	39 (75.0)	3 (75.0)	13 (61.9)

Table 1. Cont.

(b) DEJ—dermal parameters vertical												
DEJ-DERMAL PARAMETERS VERTICAL		Flattened rete ridges	Elongated rete ridges	Well defined DEJ	Less defined (dis- rupted) DEJ	Hyporeflecti- void struc- tures/nests/lo	Dark rim/ clefing	Prominent vessels	Shoal of fish pattern of the lobules	String of pearls patterns of the lobules (connected to the DEJ)	White stromal reaction (vertical)	Black ar- eas/hyporeflexive cysts inside the nests
Parameters used in logistic regression for:	BCC vs. non BCC				X	X	X					
	Superficial BCC vs. other pure BCC subtypes (nodular, fibrosing)								X	X		
Not-BCC	(n = 69)	6 (8.7)	2 (2.9)	41 (59.4)	21 (30.4)	10 (14.5)	9 (13.0)	28 (40.6)	3 (4.3)	8 (11.6)	21 (30.4)	3 (4.3)
All BCC	(n = 113)	1 (0.9)	0 (0)	26 (23.0)	107 (94.7)	107 (94.7)	88 (77.9)	54 (47.8)	26 (23.0)	56 (49.6)	64 (56.6)	19 (16.8)
Superficial BCC	(n = 35)	0 (0)	0 (0)	10 (28.6)	34 (97.1)	32 (91.4)	29 (82.9)	14 (40.0)	13 (37.1)	28 (80.0)	16 (45.7)	1 (2.9)
Nodular BCC	(n = 52)	0 (0)	0 (0)	11 (21.2)	50 (96.2)	51 (98.1)	38 (73.1)	25 (48.1)	4 (7.7)	13 (25.0)	31 (59.6)	12 (23.1)
Infiltrative BCC	(n = 4)	1 (25.0)	0 (0)	1 (25.0)	3 (75.0)	2 (50.0)	1 (25.0)	4 (100)	4 (100)	0 (0)	3 (75)	0 (0)
Mixed BCC	(n = 21)	0 (0)	0 (0)	3 (14.3)	20 (95.2)	21 (100)	19 (90.5)	11 (52.4)	5 (23.8)	15 (71.4)	14 (66.7)	6 (28.6)

Table 1. Cont.

(c) LC-OCT parameters horizontal									
PARAMETERS HORIZONTAL		Pagetoid cells in the epidermis-DEJ (horizontal)	Bright stromal reaction (horizontal)	Polarization of nuclei in the epidermis (horizontal)	Palisading (horizontal)	Clefting (horizontal)	Tumor nests/cords/silhouettes (horizontal)	Prominent vessels (horizontal)	Bright collagen alterations (elastosis)(horizontal)
Parameters used in logistic regression for:	BCC vs. non BCC			X	X	X	X		
	Superficial BCC vs. other pure BCC subtypes (nodular, fibrosing)								
Not-BCC	(n = 69)	4 (5.8)	17 (24.6)	2 (2.9)	1 (1.4)	3 (4.3)	10 (14.5)	9 (13.0)	30 (43.5)
All BCC	(n = 113)	28 (24.8)	70 (61.9)	58 (51.3)	40 (35.4)	38 (33.6)	78 (69.0)	42 (37.2)	79 (69.9)
Superficial BCC	(n = 35)	13 (37.1)	18 (51.4)	17 (48.6)	10 (28.6)	10 (28.6)	25 (71.4)	16 (45.7)	21 (60.0)
Nodular BCC	(n = 52)	11 (21.2)	34 (65.4)	28 (53.8)	23 (44.2)	23 (44.2)	37 (71.2)	16 (30.8)	43 (82.7)
Infiltrative BCC	(n = 4)	0 (0)	3 (75.0)	2 (50.0)	1 (25.0)	1 (25.0)	3 (75.0)	2 (50.0)	3 (75.0)
Mixed BCC	(n = 21)	4 (19.0)	15 (71.4)	10 (47.6)	6 (28.6)	4 (19.0)	13 (61.9)	8 (38.1)	11 (52.4)

3. Results

3.1. Population

A total of 182 lesions clinically suspicious for BCC in 154 patients were included in this study; 113 (62.1%) were confirmed histopathologically as BCCs and 69 (37.9%) as non-BCCs. The histopathological analysis entailed 35 (19.2%) completely excised lesions, 132 (72.5%) lesions acquired by punch biopsy, and 15 (8.2%) shave biopsies. Among the 113 BCCs, the nodular BCC subtype was seen most frequently in 52 (46%) lesions, followed by the superficial subtype in 35 (31%) and 4 (3.5%) infiltrative cases. Among the non-BCC cases, actinic keratosis (AK) was the most common diagnosis with a total of 24 lesions equaling a prevalence of 34.8%. Additional diagnoses included: squamous cell carcinoma (SCC), Bowen's disease, nevus, melanoma, sebaceous hyperplasia, scar tissue/fibrous papule, dermatofibroma, lentigo solaris/seborrheic keratosis, eczema, clear cell acanthoma, molluscum contagiosum, trichoblastoma, angioma, atypical fibroxanthoma, and granuloma (Figures 1–5) (Table 2).

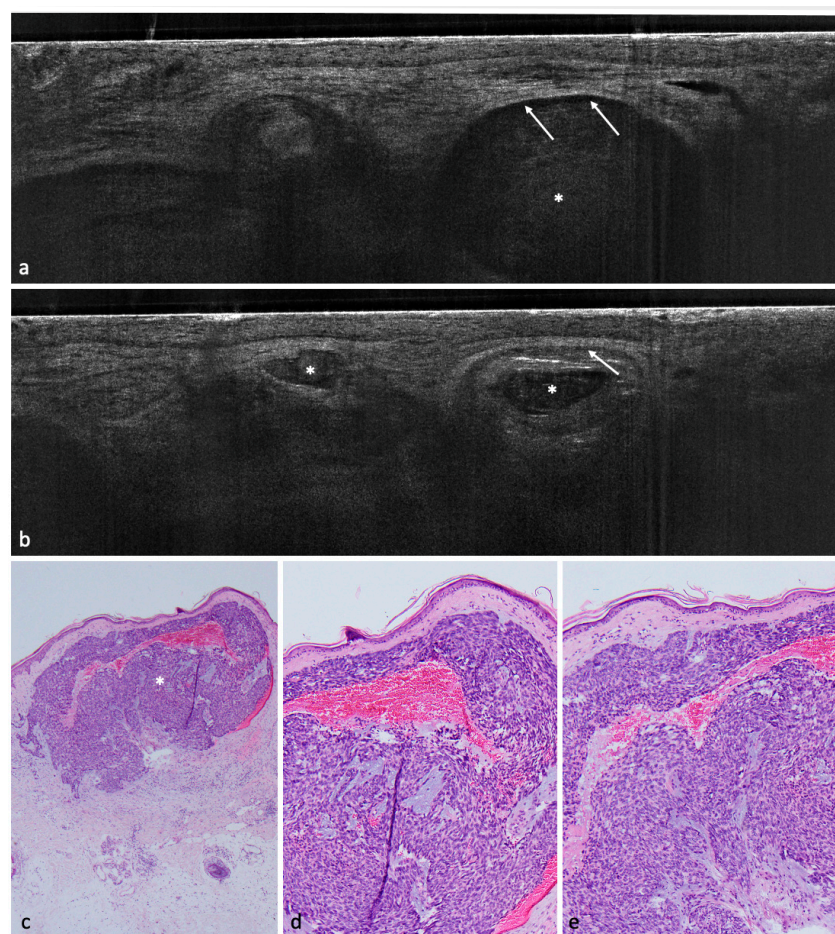


Figure 1. Nodular BCC on the lower leg of a 68-year-old female patient. (a,b) LC-OCT images. The nodular BCC presents itself with a fine granular texture corresponding to basaloid cells, peritumoral clefting (white arrows) and homogeneous areas with possibly liquefactive necrosis with remaining cell debris (white asterisks). (c) (40×), (d) (100×), and (e) (100×): corresponding histological HE-stained sections with peripheral palisading, clefting, and a central necrosis.

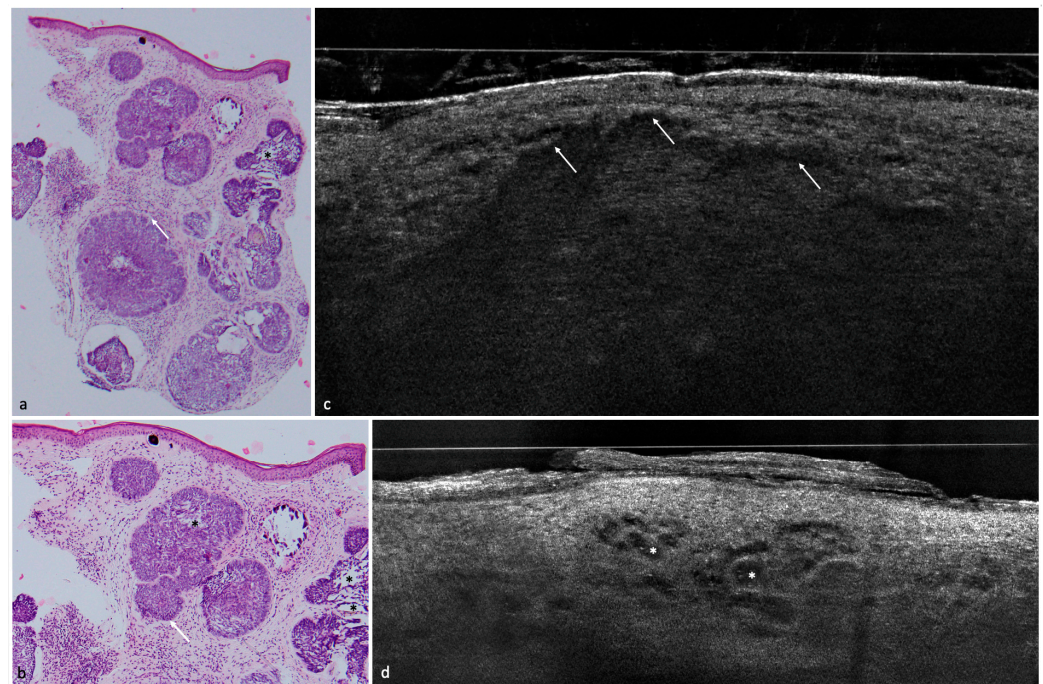


Figure 2. Nodular BCC with cystic parts in the nasolabial fold of a 76-year-old patient. (a) (200 $\times$) and (b) (100 $\times$): histological HE-stained sections. Peritumoral clefting (white arrows) and cystic structures (asterisk). (c) LC-OCT image of the same lesion. Arrows again indicate clefting, the BCC has a fine granular texture. (d) dark hyporeflective area (asterisk) in a cystic BCC corresponding to glomerular vessels.

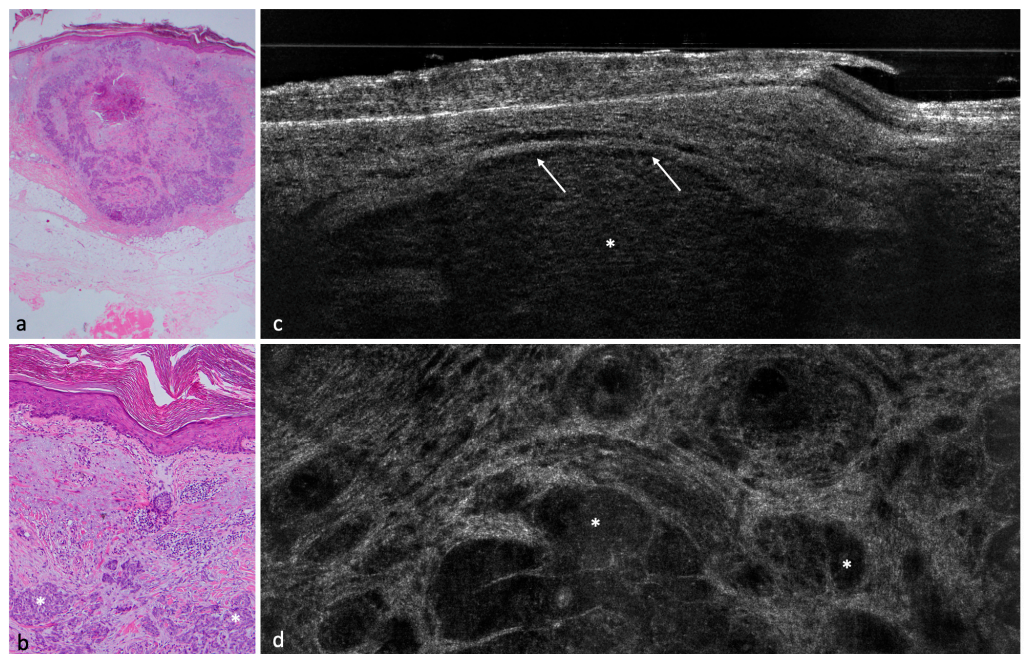


Figure 3. Nodular BCC on the right temple of a 64-year-old male patient. (a) (20 $\times$) and (b) (100 $\times$): histological HE-stained sections of the tumor. (c) (vertical) and (d) (horizontal): LC-OCT images of the corresponding lesion. Again, peripheral clefting can be seen (white arrow) as well as basaloid nests (asterisks).

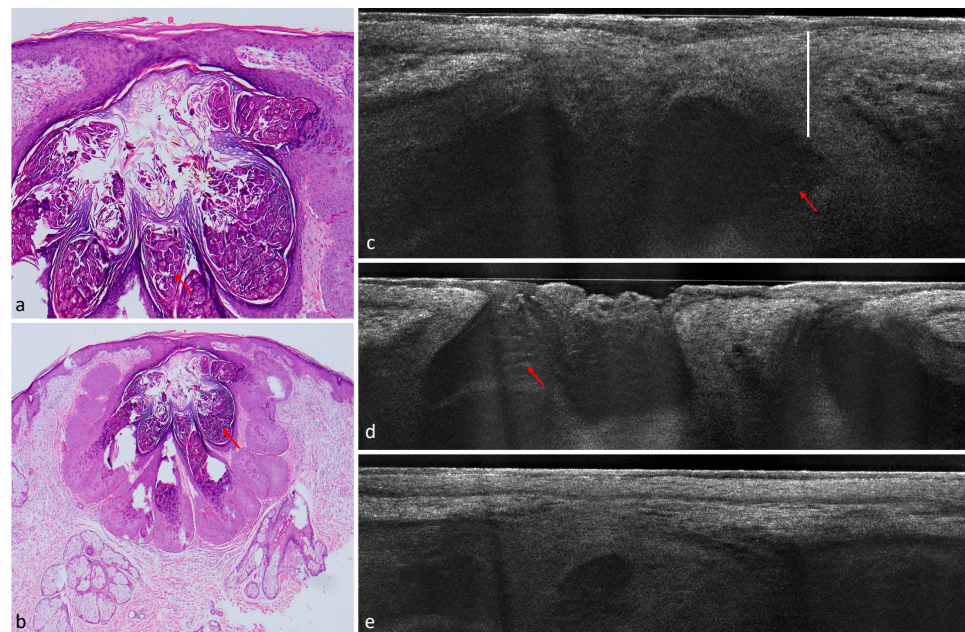


Figure 4. Molluscum contagiosum on the forehead of a 58-year-old male patient. (a) (100 $\times$) and (b) (40 $\times$): histological hematoxylin and eosin (HE)-stained sections. In histology, a cup-shaped multilobular lesion can be identified. Red arrows indicate intracytoplasmic inclusion bodies (Henderson–Paterson bodies) which contain virus particles. (c–e) LC-OCT image of the molluscum contagiosum. It shows acanthosis (white bar) and hyporefective areas with hazy structures, which probably correspond to the Henderson–Paterson bodies (red arrow).

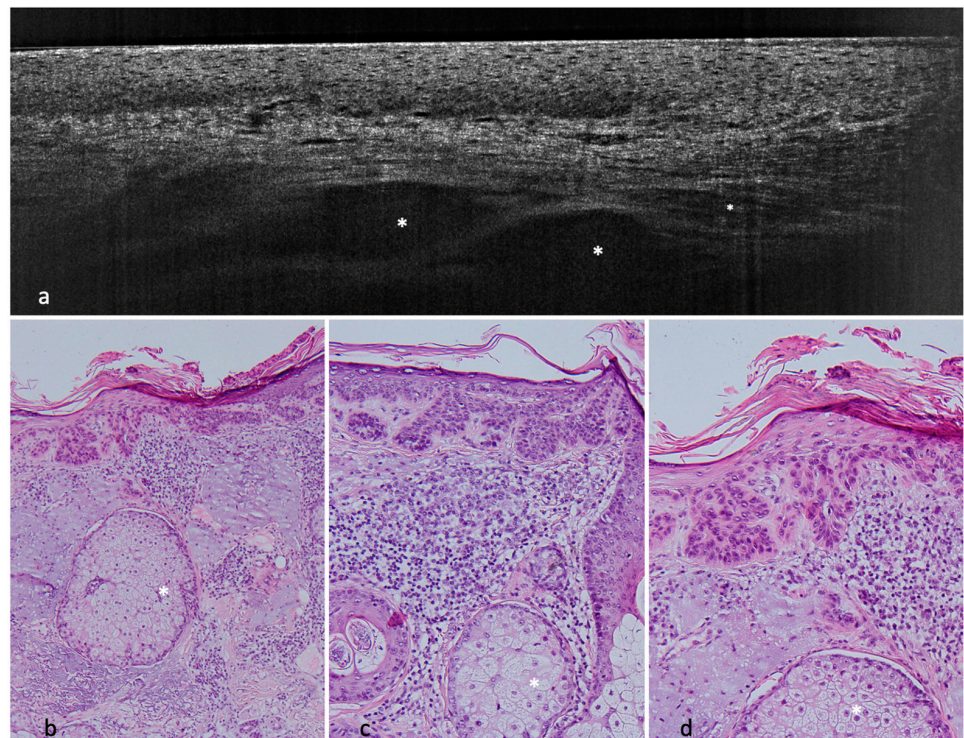


Figure 5. Sebaceous hyperplasia on the nose of a 70-year-old male patient. (a) LC-OCT image. The enlarged sebaceous glands are visible as hyporefective ovoid structures (white asterisk). When looking carefully, roundish sebocytes might be spotted within the glands. (b) (100 $\times$) and (c) (200 $\times$) and (d) (200 $\times$): histological HE-stained sections. Enlarged, regularly structured sebaceous glands (white asterisk).

Table 2. Epidemiological data.

Characteristic	Patient-Based	Lesion-Based
Mean age (+/−SD)	70.8 (12.3)	71.7 (12.0)
Sex, n(%)		
Male	102 (66.2)	
Female	52 (33.8)	
Localisation, n (%)		
Ear	1 (0.6)	1 (0.5)
Face	76 (49.4)	85 (46.7)
Head	4 (2.6)	6 (3.3)
Neck	3 (1.9)	4 (2.2)
Scalp	3 (1.9)	4 (2.2)
Trunk	49 (31.8)	62 (34.1)
Lower limb	9 (5.8)	10 (5.5)
Upper limb	9 (5.8)	10 (5.5)
Number of lesions n (%)		
1	132 (85.7)	
2	17 (11.0)	
3	4 (2.6)	
4	1 (0.6)	
Histological diagnosis, n (%)		
BCC	91 (59.1)	113 (62.1)
non-BCC	63 (40.9)	69 (37.9)
AK	21 (33.3)	24 (34.8)
SCC	8 (12.7)	9 (13.0)
Sebaceous hyperplasia	7 (11.1)	7 (10.1)
Bowen	4 (6.3)	5 (7.2)
Other non-BCC *	23 (36.5)	24 (34.8)
BCC subtypes, n (%)		
Superficial BCC	25 (27.5)	35 (31.0)
Nodular BCC	44 (48.4)	52 (46.0)
Fibrosing BCC	4 (4.4)	4 (3.5)
Nodular superficial BCC	12 (13.2)	15 (13.3)
Nodular Fibrosing BCC	2 (2.2)	3 (2.7)
Mixed	3 (3.3)	3 (2.7)

* Other non-BCC subtypes include lentigo maligna/melanoma, nevus, scar, dermatofibroma, lentigo solaris/seborrheic keratosis, eczema, clear cell acanthoma, molluscum contagiosum, fibrous papule, trichoblastoma, angioma, atypical fibroxanthoma, aspecific crust, and granuloma.

3.2. Image Quality and Diagnostic Confidence

Average LC-OCT image quality was rated as high in 162 cases (89%) and as low in 20 cases (11%); no image was considered unreadable (image quality 3).

A high diagnostic confidence (1) by at least one observer was only scored in 48% of the dermoscopic images, compared to 70% of the LC-OCT cases. The interobserver agreement on the confidence level was very good. With LC-OCT, this was the case in 173 out of 182 (95%) of the observations (weighted kappa = 0.90 (95% CI, 8.84 to 0.97), $p \sim 0$). For der-

moscopy, the number of interobserver agreements was 177 out of 182 (97.3%) observations (weighted kappa = 0.96 (95% CI, 0.92 to 0.99), $p \sim 0$).

Our average diagnostic confidence with LC-OCT was 1.7; if we consider the subgroup of lesions with high image quality (1), we reported a confidence level of 1.1 (limbs) and 1.2 (head, trunk); in the subgroup of lesions with low image quality (2), diagnostic confidence sank to 2.3 (trunk), 2.5 (head), and 2.8 (limbs). Diagnostic confidence was therefore influenced by image quality but not by anatomical site.

3.3. Diagnostic Performance

Diagnostic accuracy of dermoscopy and LC-OCT for ruling out BCCs in suspicious lesions was 88% and 91%, respectively. Concerning sensitivity and specificity compared to the gold standard histology, dermoscopy scored a sensitivity of 90% and a specificity of 86%, while LC-OCT showed a high sensitivity (98%) and a good but lower specificity (80%). Sensitivity and specificity slightly increased when image quality was high, to 93% and 99%, respectively. If considering the subgroup of LC-OCT images with a high diagnostic confidence, however (70% of the lesions), the LC-OCT performance increased significantly, with a sensitivity of 100% and a specificity of 97% compared to the gold standard histology ($p = 1$, McNemar's test).

In daily clinical practice, the combination of dermoscopy and LC-OCT enables the clinician to confidently make an almost perfect diagnosis at the bedside. The combination of both reaches an accuracy of 92.9% in all cases and an accuracy of 98.2% in confident cases, providing a certain security for the clinician and guiding further steps in the treatment process.

3.4. BCC Subtype

The diagnostic accuracy of LC-OCT for all BCC subtypes was 90%; we reported an overall sensitivity of 77% and a specificity of 96%. When only looking at cases with either a high LC-OCT quality ($n = 101$, 11% of the lesions) or lesions with a high LC-OCT confidence level ($n = 93$, 84% of the lesions), the performance of LC-OCT showed no significant difference to histology for all subtypes ($p > 0.05$, McNemar's test). Diagnostic accuracy, sensitivity, and specificity for each subtype were: superficial BCC, 90%, 77%, 96%; nodular BCC, 88%, 96%, 82%; mixed BCC, 90%, 67%, 96%. These values slightly increased when selecting only high image quality (Table 3).

If we consider only pure BCC subtypes (86 lesions), the diagnostic accuracy increased to 92%. Sensitivity and specificity were for superficial BCCs 82% and 100%, for nodular BCCs 100% and 81%, and for fibrosing BCCs 75% and 100%, respectively.

Table 3. LC-OCT performance.

(a) All lesions				
All lesions		LC-OCT		
		BCC	Non-BCC	Total
Histology	BCC	111	2	113
	Non-BCC	14	55	69
	Total	125	57	182
Dermoscopy				
All lesions		BCC	Non-BCC	Total
		BCC	Non-BCC	Total
Histology	BCC	102	11	113
	Non-BCC	10	59	69
	Total	112	70	182

Table 3. Cont.

(b) High confidence lesions				
High confidence level for at least one of the 2 observers		LC-OCT		
		BCC	Non-BCC	Total
Histology	BCC	93	0	93
	Non-BCC	1	33	34
	Total	94	33	127
Dermoscopy				
		BCC	Non-BCC	Total
Histology	BCC	61	2	63
	Non-BCC	0	24	24
	Total	61	26	87
(c) Performance for BCC				
All BCC lesions				
N (%)		111 (100)		
Global accuracy		84%		
	Superficial BCC (N = 35)	Nodular BCC (N = 51)	Fibrosing BCC (N = 4)	Mixed (N = 21)
Accuracy	90%	88%	99%	90%
Sensitivity	77%	96%	75%	67%
Specificity	96%	82%	100%	96%
p value (Mac Nemar's test)	0.23	0.03	1	0.55
Good image quality				
N (%)		101 (91)		
Global accuracy		86%		
	Superficial BCC (N = 31)	Nodular BCC (N = 49)	Fibrosing BCC (N = 2)	Mixed (N = 19)
Accuracy	91%	90%	100%	91%
Sensitivity	81%	96%	100%	68%
Specificity	96%	85%	100%	96%
P value (Mac Nemar's test)	0.51	0.11	-	0.50
High confidence level for at least one of the 2 observers				
N (%)		93 (84)		
Global accuracy		84		
	Superficial BCC (N = 31)	Nodular BCC (N = 43)	Fibrosing BCC (N = 1)	Mixed (N = 18)
Accuracy	89%	89%	100%	89%
Sensitivity	77%	95%	100%	67%
Specificity	95%	84%	100%	95%
p value (Mac Nemar's test)	0.34	0.11	-	0.75

3.5. Diagnostic Criteria

The following nine parameters were entered in the first model of the logistic regression: vertical parameters: hyperkeratosis, thinning of the epidermis, poorly defined DEJ, hyporeflexive ovoid structures, dark rim/clefting; horizontal parameters: polarization of nuclei in the epidermis, palisading, clefting, and tumor nests. Through a backward elimination approach, non-significant parameters were removed one by one so that the three most impactful parameters to discriminate between BCC and non-BCC were: poorly defined DEJ, dark rim/clefting, and hyporeflexive ovoid structures. These findings were in line with previous work [10,11].

Concerning BCC subtypes, following parameters were inserted in the logistic regression: thinning of the epidermis, shoal of fish pattern of the lobules, and string of pearls pattern of the lobules. After logistic regression, thinning of the epidermis and string of pearls were the most impactful key criteria to influence the distinction between superficial BCC and other BCC subtypes (Figures 1–3).

3.6. Other Diagnoses

With dermoscopy, 10 lesions were mistakenly classified as BCC: two AK, two sebaceous hyperplasias (Figure 5), one Bowen's disease, one SCC, one nevus, one dermatofibroma, one molluscum contagiosum (Figure 1), and one pyogenic granuloma. Eleven BCCs were instead classified as: five SCC, two AK, one lentigo maligna/melanoma, one nevus, one scar, and one dermatofibroma. Using LC-OCT, we reported 14 false positive cases: eight AK, two seborrheic keratosis, one Bowen's disease, one sebaceous hyperplasia, one molluscum contagiosum, and one pyogenic granuloma. Two cases of nodular BCCs were mistakenly diagnosed as a scar and an SCC.

Keratinocyte skin cancer (KC), consisting of actinic keratoses (AKs), squamous cell carcinoma (SCC), and Bowen carcinomas (BCs), made up 38 of the 182 suspicious lesions. The performance of LC-OCT differed on KC compared to BCCs, showing a change in sensitivity, specificity, and confidence. Compared to dermoscopy, LC-OCT was more specific but less sensitive ($N = 182$, McNemar's p value = 0.02).

4. Discussion

In this study, we aimed to analyze the diagnostic performance of LC-OCT for BCCs among clinically unclear lesions, which can be missed with dermoscopy since they display unspecific patterns. We determined our diagnostic confidence and our diagnostic accuracy, sensitivity, and specificity in comparison to dermoscopy and to the gold standard histology. Being in a real-life setting, we also focused on our capability of distinguishing superficial BCCs from other subtypes using LC-OCT, since this has a direct influence on the choice of the therapy. Moreover, we tried to identify morphological criteria specific for BCC compared to other non-BCC differential diagnoses, which could be helpful to avoid unnecessary biopsies in the case of a benign diagnosis and to direct the patient to the correct therapeutic path in the case of malignant lesions other than BCC.

4.1. BCC Morphologic Criteria and Comparison with Histology

While clinical examination and dermoscopy are able to diagnose most clear-cut BCCs, equivocal lesions are a special target of non-invasive diagnostic techniques [5]. To date, a few preliminary studies have described morphological criteria for diagnosing BCC using the novel device LC-OCT, with good histopathological correlations [10,11]. Suppa et al. examined 89 BCCs and described tumor lobules, with (in superficial BCCs) or without connection (in nodular BCCs) to the epidermis and branched lobules (in infiltrative BCCs). In our previous study [11], we reported overlapping findings and defined, analogously to OCT terminology, the presence of a string of pearls pattern in superficial BCCs and of a shoal of fish pattern in infiltrative BCCs. Moreover, we reported an overall BCC subtype agreement between LC-OCT and histology of 90.4%, with a sensitivity of 82% and a specificity of 100% for superficial BCCs.

4.2. Diagnostic Accuracy, Sensitivity, and Specificity

Our diagnostic accuracy for BCCs was very high with both dermoscopy (88%) and LC-OCT (91%); compared to the gold standard histology, LC-OCT reached a high sensitivity (98%) but a slightly lower specificity (80%) in contrast to dermoscopy (86%).

Nevertheless, LC-OCT's specificity was higher than shown in recent studies on the use of other diagnostic techniques: OCT (range: 73–75%) or RCM (range: 38–59%). Such tools have been used in similar studies to better characterize unclear lesions. Ulrich et al., for example, analyzed the sensitivity and specificity of OCT for the assisted diagnosis of

non-pigmented BCC in a similar way in a previous observational study [13]. The additional use of OCT resulted in a specificity of 75.3% (LC-OCT: 80%), a sensitivity of 95.7% (LC-OCT: 98%), and an accuracy of 87.4% (LC-OCT: 92.9%).

The combination of dermoscopy and LC-OCT reached, in our study, an accuracy of 92.9% in all cases and of 98.2% in confident cases, providing a certain security for the clinician and guiding further steps in the treatment process. Using both tools in clinical practice enables the clinician to confidently make a very accurate diagnosis at the bedside.

Dermoscopy alone scored a lower sensitivity (90%) than expected [14]. This was probably due to the setting of clinically equivocal lesions, further supported by the fact that for only 48% of the lesions examined, the clinicians had a high level of confidence in their diagnosis with dermoscopy. This finding supports the fact that the enrolled lesions were equivocal. Our confidence improved markedly (70%) using LC-OCT.

Interestingly, if we consider only lesions with a high level of diagnostic confidence with LC-OCT (the majority), sensitivity and specificity values for the diagnosis of BCC reach those of the gold standard histology (100% and 97%, McNemar's p value = 1).

4.3. Diagnostic Confidence

The abovementioned findings point out that the clinician can significantly increase diagnostic confidence after dermoscopy based on LC-OCT images and avoid a potentially unnecessary biopsy if reassured by high confidence (which was the case in most lesions in our study). The potential practical consequences are the reduction in pain and discomfort for the patient and surgery related costs and complications.

Our diagnostic confidence was not influenced by the anatomical site of the examined lesions, but was negatively influenced by the image quality. In fact, diagnostic confidence was significantly lower (1.2 vs. 2.5) in the subgroup of high vs. low image quality. This emphasizes the value of correct and standardized image acquisition protocols and required training for imaging performing personnel.

4.4. BCC Subtyping

After correctly diagnosing a BCC out of clinically equivocal lesions, the next step requires its classification into the superficial or non-superficial subtypes; in fact, a superficial BCC can be easily treated with topical drugs or further local treatments, while other more invasive subtypes should be referred to (micrographically controlled) surgery.

We identified the absence of the thinning of the epidermis and a string of pearls pattern with lobules connected to the DEJ as the most useful criteria for diagnosing superficial BCCs. The string of pearls pattern, present in 80% of our superficial BCCs, was described as hemispheric lobules (63%) or lobules connected to the epidermis (100%) in Suppa et al.'s findings. We demonstrated that we are able to use LC-OCT to distinguish between superficial and non-superficial BCC subtypes with high diagnostic accuracy. We reached a sensitivity and specificity for diagnosing superficial BCCs of 77% and 96%, respectively, increasing to 82% and 100% when considering only pure subtypes [11].

Mixed BCC subtypes are a diagnostic challenge, since their components vary in their amount and can be easily missed. However, when nodular or fibrosing components are present, the BCC becomes a surgery candidate and should not be (except for very superficial nodular components) treated with less invasive methods. Therefore, superficial BCCs could be treated non-invasively after LC-OCT without histopathological examination.

For nodular BCC detection, LC-OCT showed the highest sensitivity with 100%, whereas specificity was highest for superficial (100%) and infiltrating (100%) BCCs. However, it needs to be mentioned that for the infiltrating BCC subtype, only a few lesions ($n = 4$) were collected in the real-life setting and the category classified as mixed subtype includes nodular superficial, nodular fibrosing, and other mixed subtypes. This may be seen as a limitation of this study.

Again, the performance of LC-OCT for all subtypes including mixed subtypes showed no significant difference to histology for all subtypes ($p > 0.05$, McNemar's test) when only

looking at cases with either a high LC-OCT quality ($n = 101$, 11% of the lesions) or lesions with a high LC-OCT confidence level ($n = 93$, 84% of the lesions).

In a second step, we tried to identify with the help of logistic regressions a few key criteria able to distinguish between BCC and non-BCCs; these were: poorly defined DEJ, hyporeflexive ovoid structures or nests/lobules (Figure 3), and dark rim/clefting (Figure 1). These criteria were consistent with previous findings [10,11].

4.5. Differential Diagnosis and Related Pitfalls

Although LC-OCT is a promising tool for the bedside differential diagnosis of BCC, there still remain pitfalls. Fourteen lesions (eight actinic keratosis, two lentigo solaris/seborrheic keratosis, one Bowen's disease, one sebaceous hyperplasia, one molluscum contagiosum, one pyogenic granuloma) were misdiagnosed as BCCs.

The actinic keratosis belonged to the Bowenoid subtype, causing a roundish contour of the epidermis with atypical keratinocytes that can be confounded with a string of pearls pattern. In this case, the expert observer should pay attention to the bright hyperkeratosis and continuity of the DEJ. Analogously, tumor lobules can be easily caused by granulomas, sebaceous lobules [15], and even molluscum bodies [16]. In such cases, the roundish or polycyclic dermal lobules are very well defined and sharply contoured, and usually contain brighter granular structures (Figure 4: molluscum contagiosum, Figure 5: sebaceous hyperplasia) or fibrotic–calcific tissue (granuloma). Furthermore, the DEJ is usually preserved and is overlined by a normal epidermis, which can, however, be thinned by the dermal structures. Sebaceous hyperplasia is also usually connected to hair follicles.

One case of BCC was misdiagnosed as a scar, probably due to the hyperreflective connective tissue masking the presence of tumor lobules, while another one was classified as an SCC due to the surrounding field cancerization in an elderly patient with hyperkeratosis, keratinocyte atypia, elastosis, and collagen alterations. For this reason, particular attention is needed in such patients when performing a bedside mapping of multiple suspicious lesions.

5. Conclusions

To sum up, we were able to reach a very good diagnostic confidence and performance in distinguishing BCCs from other BCC-suspicious lesions compared to dermoscopy and the gold standard histology. The study had limitations such as the small number of non-BCC lesions and the presence of only histologically confirmed lesions. Nevertheless, we believe LC-OCT is able to support the clinician in the process of diagnosing and subtyping BCC, as a key role to optimize the diagnostic approach and the treatment. It is particularly useful to screen BCCs in the context of clinically equivocal lesions, which can be difficult to diagnose with dermoscopy only. Moreover, it is possible to screen for superficial BCCs to be treated with less invasive methods than surgery. Larger studies on specific lesion subgroups such as facial papules are needed to gain more experience in this interesting field of dermatology.

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Data Availability Statement: Fully anonymized data are available on motivated request.

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Article

Line-Field Confocal Optical Coherence Tomography: A New Tool for the Differentiation between Nevi and Melanomas?

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Simple Summary: Typical benign nevi and advanced melanomas can be easily discriminated, but there are still some melanocytic lesions where even experts are not sure about the correct diagnosis and degree of malignancy. The high penetration depth of optical coherence tomography (OCT) allows an assessment of tumor thickness of the lesion precisely, but without cellular resolution the differentiation of melanocytic lesions remains difficult. On the other hand, reflectance confocal microscopy (RCM) allows for very good morphological identification of either a nevus or a melanoma, but cannot show the infiltration depth of the lesion because of its low penetration depth. Since the new device of line-field confocal optical coherence tomography (LC-OCT) technically closes the gap between these other two devices, in this study, we wanted to examine if it is possible to differentiate between nevi and melanomas with LC-OCT, and which criteria are the most important for it.

Abstract: Until now, the clinical differentiation between a nevus and a melanoma is still challenging in some cases. Line-field confocal optical coherence tomography (LC-OCT) is a new tool with the aim to change that. The aim of the study was to evaluate LC-OCT for the discrimination between nevi and melanomas. A total of 84 melanocytic lesions were examined with LC-OCT and 36 were also imaged with RCM. The observers recorded the diagnoses, and the presence or absence of the 18 most common imaging parameters for melanocytic lesions, nevi, and melanomas in the LC-OCT images. Their confidence in diagnosis and the image quality of LC-OCT and RCM were evaluated. The most useful criteria, the sensitivity and specificity of LC-OCT vs. RCM vs. histology, to differentiate a (dysplastic) nevus from a melanoma were analyzed. Good image quality correlated with better diagnostic performance (Spearman correlation: 0.4). LC-OCT had a 93% sensitivity and 100% specificity compared to RCM (93% sensitivity, 95% specificity) for diagnosing a melanoma (vs. all types of nevi). No difference in performance between RCM and LC-OCT was observed (McNemar's p value = 1). Both devices falsely diagnosed dysplastic nevi as non-dysplastic (43% sensitivity for dysplastic nevus diagnosis). The most significant criteria for diagnosing a melanoma with LC-OCT were irregular honeycombed patterns (92% occurrence rate; 31.7 odds ratio (OR)), the presence of pagetoid spread (89% occurrence rate; 23.6 OR) and the absence of dermal nests (23% occurrence rate, 0.02 OR). In conclusion LC-OCT is useful for the discrimination between melanomas and nevi.

Keywords: melanoma; nevus; skin cancer; line-field confocal optical coherence tomography; optical coherence tomography; reflectance confocal microscopy; skin imaging; non-invasive diagnostics in dermatology



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1. Introduction

Optical coherence tomography (OCT) and reflectance confocal microscopy (RCM) already play important roles in routine non-invasive skin cancer diagnosis [1]. OCT is well established for non-melanoma skin cancer (NMSC), whereas RCM is most commonly used for pigmented lesions [2–4]. With OCT it is possible to create vertical images until mid to deep dermal level, to reconstruct 3D images, measure tumor thickness, monitor topically treated lesions over the course of time, and even to distinguish BCC subtypes, but lacks cellular resolution [2,3]. This is why melanocytic lesions cannot be visualized very well with OCT. With the high resolution of RCM, pigmented tumors can be examined horizontally in cellular detail. Since the penetration depth of RCM is limited, it is only possible to measure from the stratum corneum to the stratum papillare of the dermis [4,5].

The new line-field confocal optical coherence tomography (LC-OCT) method offers the combination of the advantages of both devices—a high penetration depth like OCT and a similar high resolution like RCM, together with the visualization of images in horizontal and vertical views [6–11].

Therefore, LC-OCT was used in this study to investigate melanocytic lesions and to find out the most common and most useful parameters for diagnosis, especially for the differentiation between (dysplastic) nevi and melanomas. The improved distinction between nevus and melanoma with LC-OCT allows a fast, in vivo, and non-invasive diagnosis. If the lesion can be categorized as a nevus immediately with LC-OCT, no excision is necessary. In addition to the benefit to the patient that no invasive procedure is needed, this new technique will help to avoid unnecessary surgeries, leading to lower healthy insurance costs, as well as to offering more free capacities for other necessary melanoma surgeries. This study assesses the influence of image quality on diagnostic performance with LC-OCT and RCM and determines the potential of LC-OCT (vs. RCM vs. histology) to identify a melanoma vs. a nevus reliably and accurately. To our knowledge, this is the first work about melanocytic lesions, especially concerning the differentiation of nevi and melanomas with the new LC-OCT device.

2. Materials and Methods

2.1. LC-OCT

The LC-OCT device used in our study is based on a time-domain-OCT (TD-OCT), which takes several A-scans from the skin's surface down to a maximal depth of 500 μm for the acquisition of B-scans, while constantly refocusing. It has an axial resolution of 1.1 μm and a lateral resolution of 1.3 μm . The LC-OCT technique consists of a two-beam interference microscope, with a laser source of 800 nm wavelength with a continuous spectrum and a line camera as a photodetector. The laser classification is 1 M according to EN 60825-1. Like in conventional OCT, the images are depicted in grey scale. The CE-marked LC-OCT deepLive™ (DAMAE Medical, Paris, France) is a mobile central unit with a monitor and a handheld probe. To generate high quality images and reduce the optical index between the glass plate of the probe and skin layers, paraffin oil was used. The device is non-invasive, painless, and is applied without pressure. LC-OCT has three available modes to create images in real time: vertical (en-coupe) sections, as in OCT and histology, horizontal (en-face) images as in RCM, and 3D images. The field of view for both the vertical and horizontal sections is 1.2 mm $\times$ 0.5 mm. The 3D images can be taken as horizontal stacks from the top of the skin with steps of 1 μm . Short videos can also be acquired with up to 26, 16, or 8 frames/s (basic, high definition, ultra-high definition). Moreover, a dermoscopic image (resolution 5 μm , field of view 2.5 mm) is taken simultaneously, which makes it possible to navigate in the lesion and choose the correct position. Captured images can be exported and saved globally or as a single patient/lesion file in TIFF, DICOM, or JPEG formats. The additional program 3DSlicer (The Slicer Community, Open-Source Software) allows the reconstruction of 3D cubes of the horizontal LC-OCT stacks [9,10].

2.2. RCM and OCT

In our study we used commercially distributed RCM devices (VivaScope® 1500 and VivaScope® 1500/3000 Combo, Mavig GmbH, Munich, Germany), which have a penetration depth of 300 µm. Both have a lateral resolution of 1 µm and an axial resolution between 3–5 µm. The light source is an 830 nm diode laser. The 1500 device has an integrated dermoscopic camera for navigation, creates image sizes of 500 µm × 500 µm, and builds mosaics of single greyscale pictures up to 8 mm × 8 mm (VivaBlock®). The other mode, which we used in the study for the comparison with LC-OCT, is the VivaStack®, where single images can be taken from the skin's surface to the stratum papillare in several steps. The conventional OCT used in this study is the commercially available, handheld-based OCT device VivoSight® (Michelson Diagnostics Ltd., Maidstone, Kent, UK). We additionally measured 30 of all the melanocytic lesions with conventional OCT for comparative reasons. The OCT acquires images of 6 mm × 6 mm with 1.5 mm detection depth. It has a lateral resolution of 7.5 µm and an axial resolution of 10 µm, and contains a laser source of 1305 nm. With the addition of dynamic OCT, blood flow and blood vessels can be visualized. Further details on the devices are described elsewhere [2,12].

2.3. Patients

Patients with suspicious melanocytic lesions or nevi that were planned for excision were enrolled in the study at the Department of Dermatology and Allergology at the University Hospital of Augsburg and at the University Hospital of the Ludwig Maximilian University, Munich in Germany between November 2019 and January 2021. The study was approved by the local ethics committee (No. 17-699) and conducted according to the principles of the Declaration of Helsinki and international guidelines concerning human studies. Written informed consent was obtained from all patients prior to inclusion into the study.

2.4. Measurements

A clinical examination was done to identify the lesion as suspicious for a melanoma or a (dysplastic) nevus prior to excision. Clinical and dermoscopic images of each lesion were acquired using Fotofinder® (FotoFinder Systems GmbH, Bad Birnbach, Germany), Dermo-Genius 2® (DermoScan GmbH, Regensburg, Germany), ILLUCO IDS-1100 (DermoScan GmbH, Regensburg, Germany), or an iPhone 12 Pro Camera (Apple Inc., Cupertino, CA, USA). After dermoscopy, the lesions were scanned with LC-OCT (deepLive™, DAMAE Medical, Paris, France) in horizontal, vertical, and 3D. After that, OCT images (vertical and dynamic en-face) as well as RCM pictures were taken (VivaStacks® and VivaBlocks®). Horizontal LC-OCT and RCM images were taken at the level of the stratum corneum, the epidermis, the dermo-epidermal junction (DEJ), and the papillary dermis. For measurements with RCM and LC-OCT, a few drops of paraffin oil were used before the scan. OCT examinations did not require preparation of the skin. After the examination, a biopsy, shave, or excision of the lesion was taken and sent for histological analysis. All lesions were compared with standard histology in haematoxylin/eosin.

After all images had been reviewed, the observers graded LC-OCT and RCM image quality during a consensus meeting with semi-quantitative scores as poor (3), acceptable (2), good (1), or excellent (0), and the confidence level from low (3), medium (2), high (1), to very high (0). After histology, the final diagnosis was noted. Both centers are regular users of OCT, RCM, and LC-OCT. Both centers had at least 3 months of practical experience and training with LC-OCT before the study.

In addition to the well-known clinical and dermoscopic diagnostic criteria for melanocytic lesions, we used the following patterns for the different methods: LC-OCT criteria in horizontal and vertical view (see Tables S1 and S2) and RCM parameters (see Tables S3 and S4). In 30 cases, conventional OCT images were also recorded for demonstration purposes. Since RCM is superior to OCT in diagnosing melanocytic lesions and OCT cannot show the

cellular details of melanomas and nevi, OCT criteria were not evaluated and OCT was not included in the systematic study [13,14].

2.5. Statistical Analysis

For the collection of data, Microsoft® Office Excel® for Mac 2021 was used. Statistical analysis was performed with R version 4.0.0 (R Foundation for Statistical Computing; Vienna, Austria). The accuracy, specificity, sensitivity of each technique, PPV (percentage of positive diagnoses that were correct), and NPV (percentage of negative diagnoses that were correct) were calculated. The specificity and sensitivity of LC-OCT for the diagnosis of a melanoma were compared with the specificity and sensitivity of RCM, and then both devices with histology using McNemar's test, which considers the paired nature of the data. Furthermore, the investigators' responses concerning the recognized parameters for melanocytic lesions, the diagnoses, the image quality, and confidence level were noted. For the intra-method correlation between image quality and confidence level, Spearman's correlation coefficients (r) were calculated. A p -value < 0.05 was considered as statistically significant. When the data failed the normality test, a Wilcoxon test for paired tests was used to compare image quality and confidence level between LC-OCT and RCM. We also performed univariate and multivariate logistic regression analyses for the criteria that were more useful in discriminating a melanoma from a nevus (dysplastic or not), and used a backward elimination approach.

3. Results

3.1. Study Population

A total of 75 patients with 84 melanocytic lesions were evaluated with LC-OCT before excision. A total of 36 of the 84 lesions were also measured with RCM. Five patients had two lesions, one patient had five lesions, and the others had one pigmented lesion. The mean age of the patients was 51 years. Lesions were mainly located on the trunk (57.1%), limb (25%), and the head (17.9%). Histology was available for all 84 lesions. Histology identified 42 (50%) of 84 lesions as banal nevi, 13 (15.5%) as dysplastic nevi and 28 (33.3%) as melanomas. A total of 65.5% of the lesions were histologically diagnosed as nevi (dysplastic or not). Furthermore, 82.1% of the lesions were completely excised, 16.7% were biopsied, and 1.2% were shaved. One lesion was histologically diagnosed as a pigmented actinic keratosis and was therefore excluded for further evaluation. In terms of invasion levels, nine melanomas were in situ melanomas (32%), and nine invasive melanomas were up to 1 mm thick (32%). A total of seven melanomas had a tumor thickness between 1 and 2 mm (25%), two melanomas were between 2 and 4 mm thick (7%), and one melanoma was thicker than 4 mm (4%). Regarding the subtype, 17 melanomas were classified as superficial spreading melanomas (61%), 2 were nodular (7%), 2 were acrolentiginous (7%), and 7 lentigo maligna (3) or invasive lentigo maligna melanomas (4) (25%). TNM classifications for the melanomas were T0 (9), IA (9), IB (7), IIA (1), IIB (1), and IIIC (1). All melanomas with a tumor thickness up to 2 mm were excised with a safety margin of 1 cm, while melanomas with a tumor thickness ≥ 2 mm received excision with a safety margin of 2 cm. In patients with a melanoma with a tumor thickness > 1 mm, a sentinel lymph node biopsy (or in high-risk cases with a tumor thickness > 0.75 mm) was conducted.

3.2. LC-OCT and RCM Image Quality and Confidence Level

A total of 1 out of 36 lesions imaged with RCM and 4 from 84 lesions imaged with LC-OCT were classified as having poor image quality. The diagnostic confidence level depended on image quality for LC-OCT and RCM (Table 1, Figure 1).

Lower image quality led to a poorer confidence level (Table 2). We found no difference between RCM and LC-OCT in terms of average image quality or average diagnostic confidence level (Table 2). A total of 77.8% of the lesions had good LC-OCT image quality (scored 0 or 1), while 72.2% had good RCM image quality. The average quality was evaluated as 1.2 for LC-OCT vs. 1.3. for RCM. However, the image quality ($p = 0.49$) and

the confidence level ($p = 0.40$) were not significantly different between LC-OCT and RCM ($p = 0.49$). In total, 86.1% of the lesions had a high LC-OCT confidence level (scored 0 or 1), while 77.8% had a high RCM confidence level. The average confidence level was calculated as 1.2 for LC-OCT vs. 1.2 for RCM.

Table 1. Influence of image quality on the LC-OCT and RCM confidence level. LC-OCT and RCM image quality was scored from 0 (higher quality) to 3 (lower quality). LC-OCT and RCM confidence level was scored from 0 (higher confidence level) to 3 (lower confidence level).

LC-OCT				
For all lesions ($n = 84$)	Quality = 0 ($n = 4$)	Quality = 1 ($n = 58$)	Quality = 2 ($n = 18$)	Quality = 3 ($n = 4$)
Average confidence level	0.8	1.2	1.4	2.3
For lesions also imaged with RCM ($n = 36$)	Quality = 0 ($n = 1$)	Quality = 1 ($n = 27$)	Quality = 2 ($n = 7$)	Quality = 3 ($n = 1$)
Average confidence level	1	1.1	1.3	3
RCM				
$n = 36$	Quality = 0 ($n = 0$)	Quality = 1 ($n = 26$)	Quality = 2 ($n = 9$)	Quality = 3 ($n = 1$)
Average confidence level	-	1.1	1.4	2

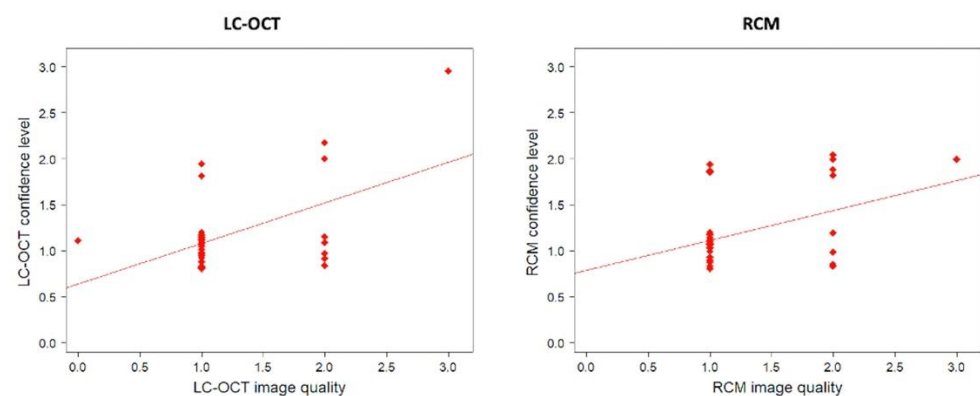


Figure 1. Correlation between confidence level and image quality for LC-OCT and RCM. There was a statistically significant positive correlation between LC-OCT confidence levels and image quality for LC-OCT ($r_s(36) = 0.40$; $p = 0.02$), as well as for RCM ($r_s(36) = 0.43$; $p = 0.008$).

Table 2. Comparison of image quality and confidence level between LC-OCT and RCM.

	LC-OCT	RCM
Image quality	n (%)	n (%)
Score 0	1 (2.8)	0 (0)
Score 1	27 (75.0)	26 (72.2)
Score 2	7 (19.4)	9 (25.0)
Score 3	1 (2.8)	1 (2.8)
Confidence level	n (%)	n (%)
Score 0	0 (0)	0 (0)
Score 1	31 (86.1)	28 (77.8)
Score 2	4 (11.1)	8 (22.2)
Score 3	1 (2.8)	0 (0)

3.3. LC-OCT and RCM Performance for Diagnosing a Melanoma vs. a Nevus

Table 3 shows LC-OCT performances for diagnosing a melanoma vs. a nevus (dysplastic or not), and Table 4 for diagnosing a melanoma vs. a nevus vs. a dysplastic nevus for all 84 lesions.

Table 3. LC-OCT performances for diagnosing melanomas vs. nevi (dysplastic or not) compared to histology.

All Lesions		Histology			Total
		Melanoma	Nevus	Other	
LC-OCT	Melanoma	26	0	0	26
	Nevus	2	55	0	57
	Other	0	0	1	1
	Total	28	55	1	84

Table 4. LC-OCT performances for diagnosing melanomas vs. nevi vs. dysplastic nevi compared to histology.

All Lesions		Histology				Total
		Nevus	Melanoma	Dysplastic Nevus	Others	
LC-OCT	Nevus	40	1	8	0	49
	Melanoma	0	26	0	0	26
	Dysplastic nevus	2	1	5	0	8
	Others	0	0	0	1	1
	Total	42	28	13	1	84

Figure 2 shows an example of a compound nevus, Figure 3 a dysplastic nevus and Figure 4 a melanoma with all three devices compared to histology. The case diagnosed as “other” (pigmented actinic keratosis) in histology was dropped for the evaluation.

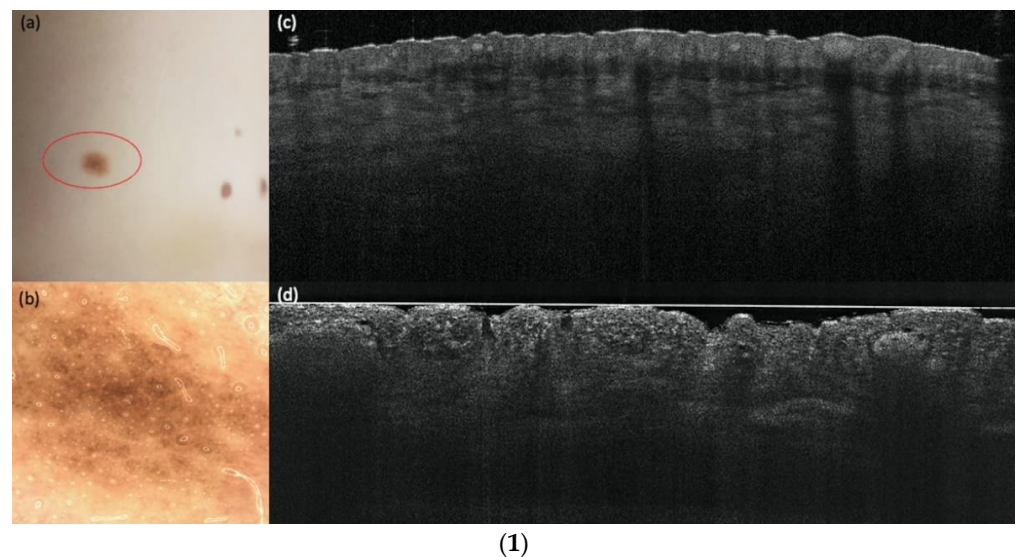


Figure 2. Cont.

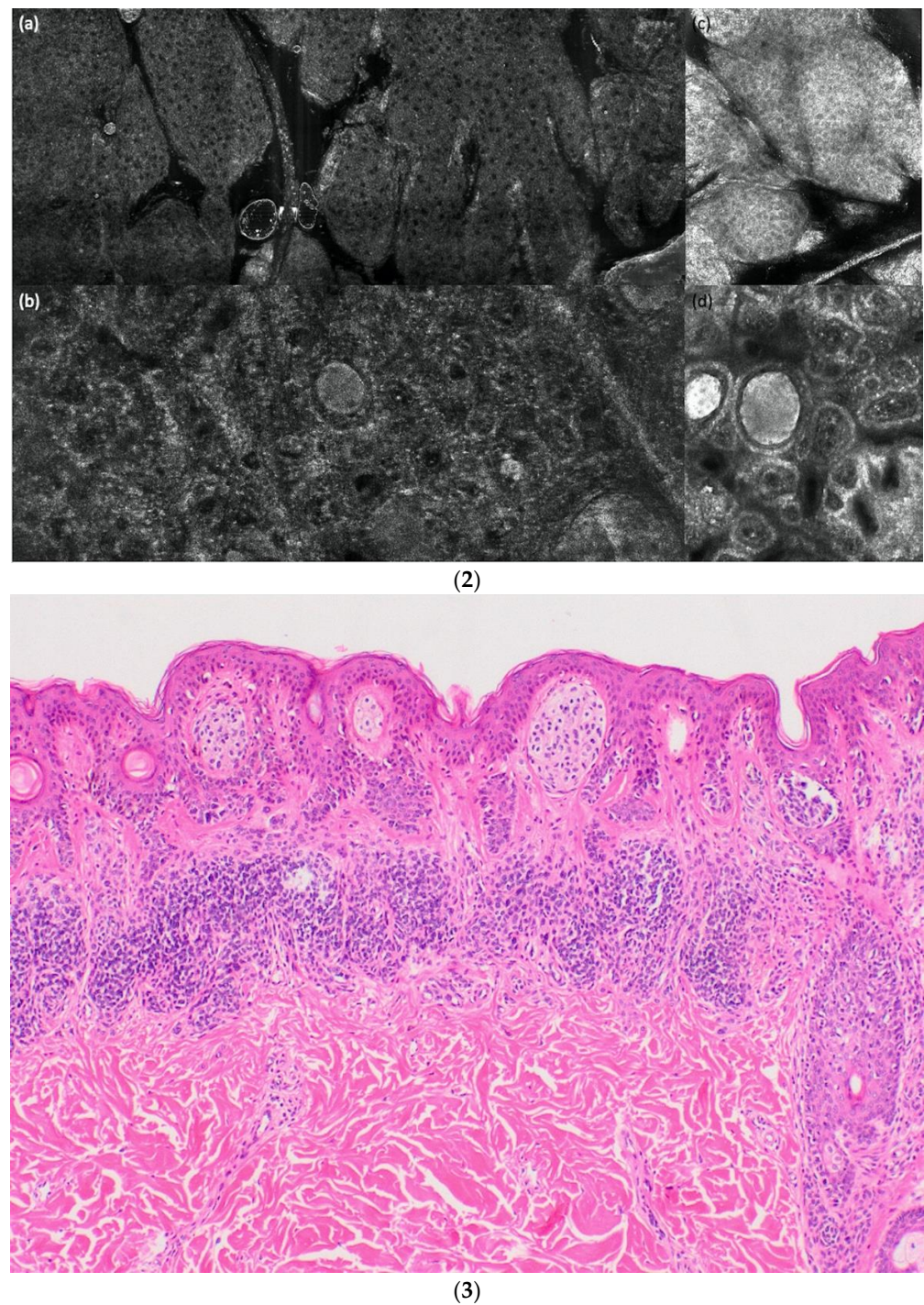


Figure 2. (1) A compound nevus in comparison with vertical LC-OCT and OCT. A compound nevus on the left lumbar trunk. (a) Clinical, (b) dermoscopic, and (c) optical coherence tomography (OCT; 6 mm × 2 mm) images of a representative nevus of the study. (d) In vertical line-field optical coherence tomography (LC-OCT; 1.2 mm × 0.5 mm) images junctional and dermal nests, a well-defined dermo-epidermal junction (DEJ), and a papillomatous surface can be seen. (2). A compound nevus in comparison with horizontal LC-OCT and RCM. The same compound nevus is depicted in horizontal LC-OCT (1.2 mm × 0.5 mm) with a regular honeycomb pattern (a), with nests in the upper dermis and regular papillae (b). Reflectance confocal microscopy (RCM; 500 μm × 500 μm) shows the same features. A regular honeycomb pattern (c) and junctional nests, as well as regular papillae, but just a little bit brighter (d). (3). A compound nevus in histology. The histology shows the same compound nevus as in (1) and (2) with 4× magnification.

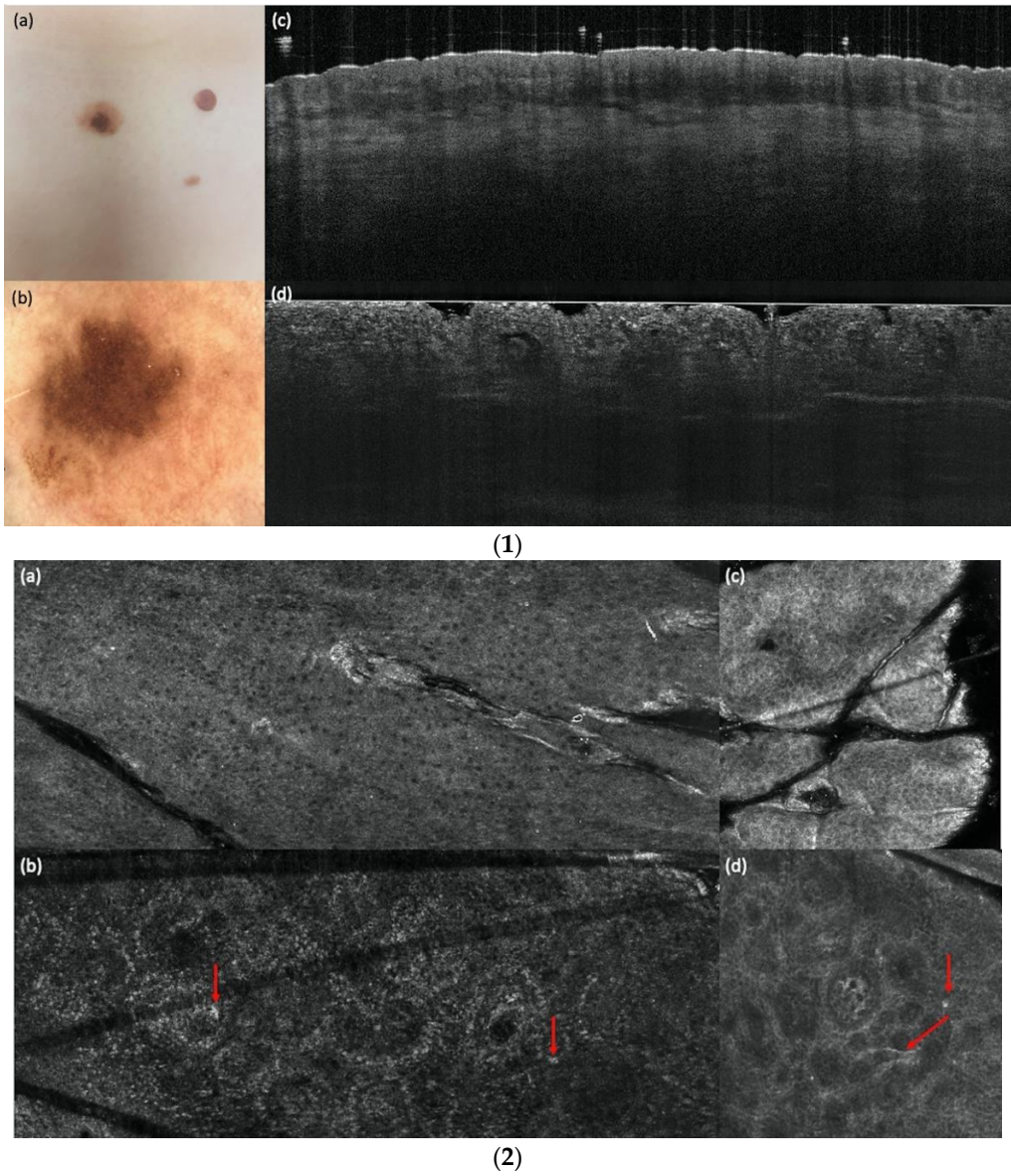


Figure 3. Cont.

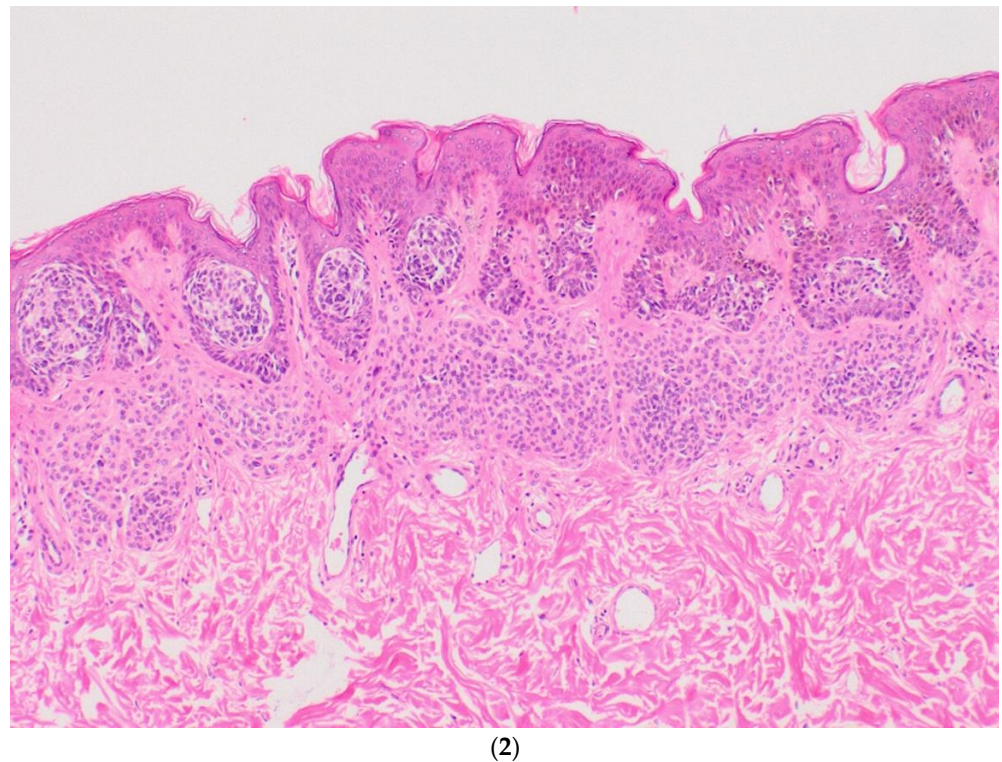


Figure 3. (1). A dysplastic compound nevus in comparison with vertical LC-OCT and OCT. A dysplastic nevus on the left lower trunk. (a) Clinical, (b) dermoscopic, and (c) optical coherence tomography (OCT; 6 mm × 2 mm) images of a representative dysplastic nevus of the study. (d) In vertical line-field optical coherence tomography (LC-OCT; 1.2 mm × 0.5 mm) images, junctional and dermal nests, a well-defined dermo-epidermal junction (DEJ), but also a few bright atypical cells are visible. (2) A dysplastic compound nevus in comparison with horizontal LC-OCT and RCM. The same dysplastic compound nevus is depicted in horizontal LC-OCT (1.2 mm × 0.5 mm) with a regular honeycomb pattern (a), with nests in the upper dermis, less regular papillae, and a few bright atypical cells (red arrows) (b). Reflectance confocal microscopy (RCM; 500 μm × 500 μm) shows the same features. A regular honeycomb pattern (c) and junctional nests, less regular papillae, and also a few bright atypical cells (red arrows) (d). (3) A dysplastic compound nevus in histology. The histology shows the same compound nevus as in (1) and (2) with 4× magnification.

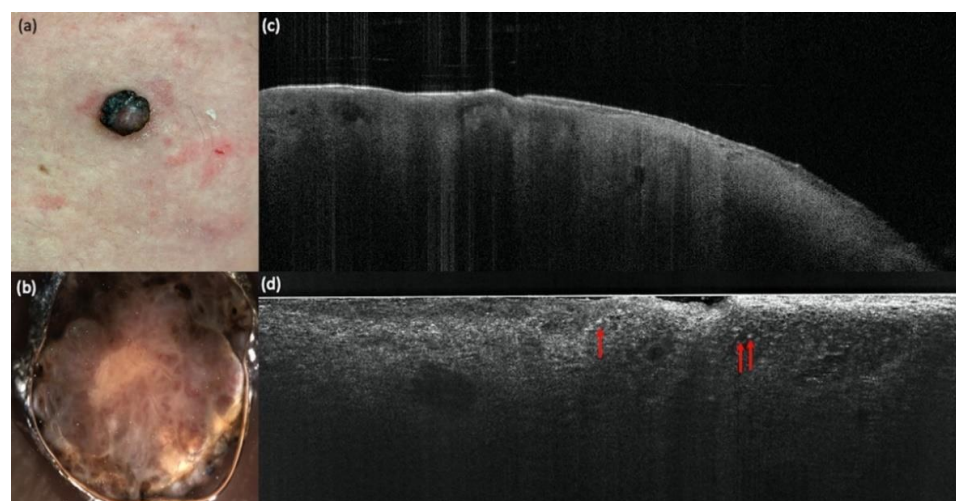


Figure 4. Cont.

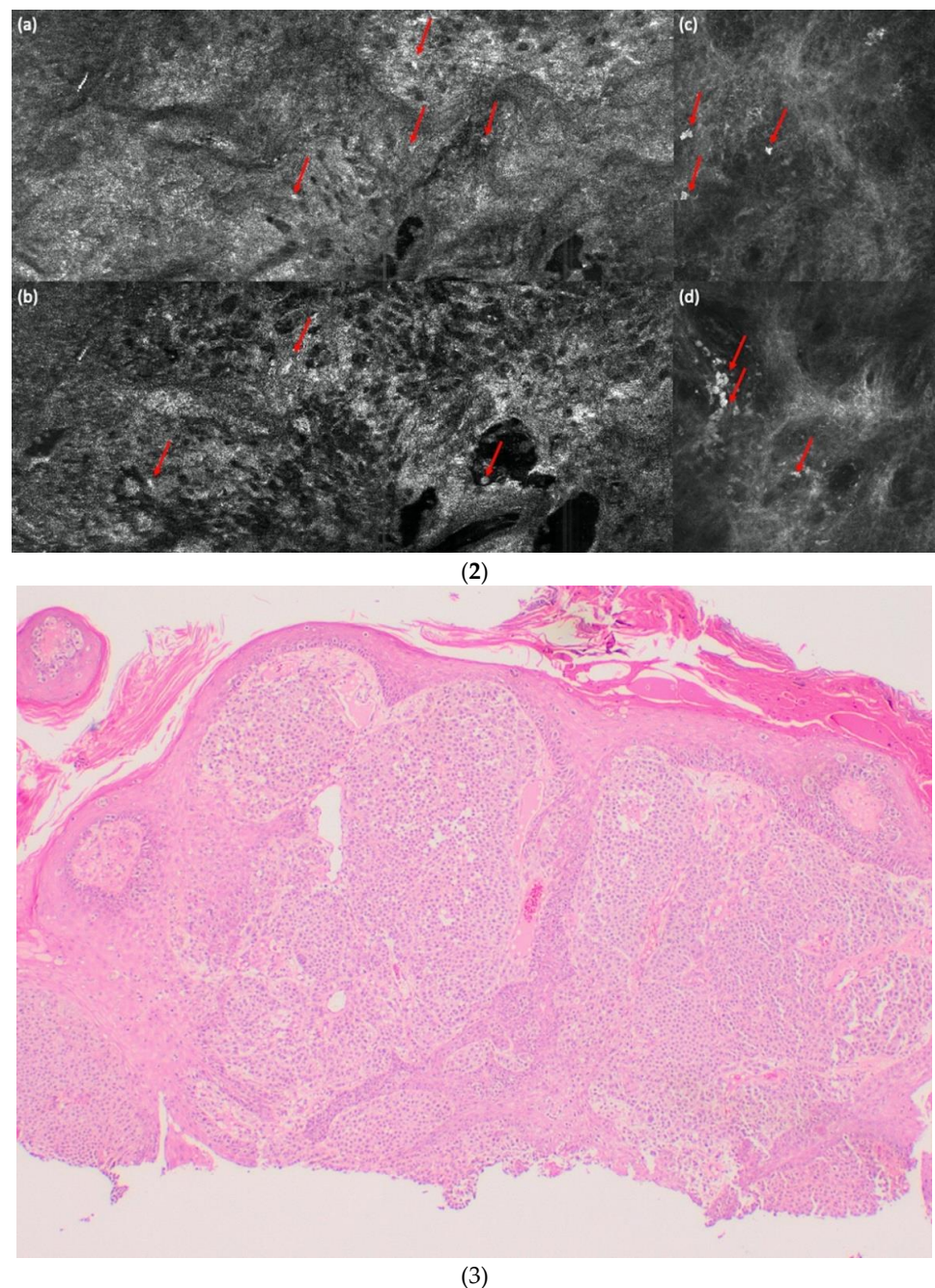


Figure 4. (1) A melanoma in comparison with vertical LC-OCT and OCT. An ulcerated nodular melanoma on the upper right leg. (a) Clinical, (b) dermoscopic, and (c) optical coherence tomography (OCT; 6 mm × 2 mm) images of a representative melanoma of the study. (d) In vertical line-field optical coherence tomography (LC-OCT; 1.2 mm × 0.5 mm) images, bright atypical melanocytic cells (red arrows) and a disturbed dermo-epidermal junction (DEJ) are visible. No dermal nests can be seen. (2) A melanoma in comparison with horizontal LC-OCT and RCM. The same melanoma is depicted in horizontal LC-OCT (1.2 mm × 0.5 mm) with an irregular honeycomb pattern with single atypical cells (red arrows) (a), and a pagetoid spread with atypical melanocytes (red arrows) (b). There were no dermal nests, a more chaotic structure, and no edged papillae visible. Reflectance confocal microscopy (RCM; 500 μm × 500 μm) shows the same features. An irregular honeycomb pattern with single atypical cells, just brighter (red arrows), (c) and a lighter pagetoid spread with atypical melanocytes (red arrows) (d). There were also no dermal nests, a more chaotic structure, and no edged papillae visible. (3) A melanoma in histology. The histology shows the same ulcerated nodular melanoma as in (1) and (2) with a tumor thickness of 4.8 mm and with 4× magnification.

The accuracy of all LC-OCT performances for diagnosing a melanoma vs. nevus was 97.6%, the sensitivity was 92.9%, and the specificity was 100% (see Table 5). The specificity and sensitivity of LC-OCT for diagnosing a melanoma vs. a nevus were compared with the specificity and sensitivity of histology using McNemar's test ($p = 0.48$). Two melanomas were falsely negative and were diagnosed as nevi with LC-OCT (for the analysis, see Table S5).

Table 5. LC-OCT performances for diagnosing melanomas vs. nevi (dysplastic or not).

	<i>n</i>	Global Accuracy	Nevus (<i>n</i> = 42)			Dysplastic Nevus (<i>n</i> = 13)			Melanoma (<i>n</i> = 28)		
			Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity
LC-OCT	83	86%	87%	95%	78%	87%	38%	96%	98%	93%	100%

Tables 6–8 illustrate the subgroup of 36 lesions that were imaged with LC-OCT and RCM. The accuracy of all performances with LC-OCT was 97.1% vs. 94.3% with RCM, the sensitivity for both was 92.9%, and the specificity with LC-OCT was 100% vs. 95.2% with RCM. The specificity and sensitivity of both devices for diagnosing a melanoma vs. a nevus were compared with the specificity and sensitivity of histology and with each other using McNemar's test (for all $p = 1$). One false negative was found, which was the same lesion for LC-OCT and RCM, and which had a bad image quality score in LC-OCT (3) and in RCM (2).

Table 6. LC-OCT and RCM performances for diagnosing melanomas vs. nevi (dysplastic or not) compared to histology.

<i>n</i> = 36		Histology			
		Melanoma	Nevus	Other	Total
LC-OCT	Melanoma	13	0	0	13
	Nevus	1	21	0	22
	Other	0	0	1	1
	Total	14	21	1	36
RCM	Melanoma	13	1	1	15
	Nevus	1	20	0	21
	Other	0	0	0	0
	Total	14	21	1	36

Table 7. LC-OCT and RCM performances for diagnosing melanomas vs. nevi vs. dysplastic nevi compared to histology.

<i>n</i> = 36		Histology				
		Nevus	Melanoma	Dysplastic Nevus	Others	Total
LC-OCT	Nevus	14	1	4	0	19
	Melanoma	0	13	0	0	13
	Dysplastic nevus	0	0	3	0	3
	Others	0	0	0	1	1
	Total	14	14	7	1	36
RCM	Nevus	13	0	3	0	16
	Melanoma	0	13	1	1	15
	Dysplastic nevus	1	1	3	0	5
	Others	0	0	0	0	0
	Total	14	14	7	1	36

Table 8. LC-OCT and RCM performances for diagnosing melanomas vs. nevi vs. dysplastic nevi.

	<i>n</i>	Global Accuracy	Nevus (<i>n</i> = 14)			Dysplastic Nevus (<i>n</i> = 7)			Melanoma (<i>n</i> = 14)		
			Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity
LC-OCT	35	86%	86%	100%	76%	89%	43%	100%	97%	93%	100%
RCM	35	83%	89%	93%	86%	83%	43%	93%	94%	93%	95%

The most significant criteria for diagnosing a melanoma with LC-OCT were irregular honeycombed pattern (92% occurrence rate; 31.7 OR), presence of pagetoid spread (89 % occurrence rate; 23.6 OR) and absence of dermal nests (23 % occurrence rate, 0.02 OR) as seen in Table 9.

Table 9. LC-OCT and RCM key criteria that were more useful in discriminating a melanoma from a nevus (dysplastic or not).

LC-OCT Parameters	OR (Univariate)	<i>p</i> Value	OR (Multivariate)	<i>p</i> Value
Horizontal parameters				
Irregular honeycombed pattern	43.64 (10.80–299.67)	<0.001	18.03 (1.50–547.32)	0.039
Pagetoid spread with atypical melanocytes in basal/suprabasal layers	41.21 (11.28–206.45)	<0.001	16.56 (1.43–435.95)	0.037
Edged papillae	0.22 (0.08–0.59)	0.003	0.43 (0.02–9.05)	0.57
Basal nests	0.23 (0.07–0.63)	0.007	0.51 (0.02–12.06)	0.67
Nests in the upper dermis	0.21 (0.06–0.61)	0.007	0.23 (0.01–3.80)	0.35
Irregular bright cells/sheets of cells in the upper dermis	5.75 (1.77–20.94)	0.005	0.51 (0.02–10.73)	0.67
Vertical parameters				
Pagetoid spread of bright cells in suprabasal/basal layers	20.36 (6.44–74.84)	<0.001	4.74 (0.35–94.88)	0.25
Junctional nests	0.31 (0.11–0.83)	0.02	1.85 (0.06–103.11)	0.73
Disturbed DEJ	10.83 (3.73–35.72)	<0.001	4.85 (0.26–144.46)	0.31
Dermal nests	0.21 (0.07–0.58)	0.004	0.08 (0.00–1.29)	0.12
Sheets of atypical bright cells	26.47 (4.50–506.78)	0.003	0.69 (0.02–29.63)	0.83
RCM parameters				
Irregular honeycombed pattern	69.67 (9.13–1553.6)	<0.001	123.91 (2.84–19309)	0.030
Pagetoid spread with atypical melanocytes in suprabasal layers	34.00 (5.91–315.56)	<0.001	0.76 (0.01–23.85)	0.88
Edged papillae	0.08 (0.01–0.44)	0.007	0.05 (0.00–0.76)	0.053

4. Discussion

Due to the high resolution and good penetration depth of LC-OCT, it is finally possible to overcome the gap between OCT and RCM. The vertical view is similar to OCT, and visualizes single cells, making the comparison with histology very intuitive. Monnier et al. already proved that LC-OCT can discriminate different skin levels and keratinocytes in healthy skin acquisitions [15–17]. Recent studies show that with single cell display, even BCC subtypes can be discriminated in vertical LC-OCT [18]. The disadvantage of LC-OCT is the lower penetration depth compared to OCT, so it is possible that deeper tumor parts may be missed [19,20]. Due to the presentation of single cells, differential diagnoses such as sebaceous hyperplasia, actinic keratoses, and squamous cell carcinomas could be differentiated, and the proliferation degree of actinic keratoses showed a 75% concordance between LC-OCT and histology [21–24].

This high single cell resolution is the reason why we assumed that even melanocytic lesions can be evaluated with LC-OCT. Regarding resolution, the horizontal LC-OCT images were very similar to RCM, which is the reference standard. With RCM it has already been shown that nevi can be discriminated from melanomas, even if there are sometimes possible false-negative and false-positive cases of melanomas [25]. Moreover, ex vivo RCM has also been successfully used for the differentiation and diagnosis of melanocytic lesions, although not all typical in vivo features could be detected since it offers a vertical view instead of horizontal as in in vivo RCM [26]. Hartmann et al. stated that ex vivo RCM might also be useful in the measurement of tumor thickness, and therefore might be of help for the presurgical definition of correct margins [27]. LC-OCT is similar to a fusion of ex vivo and in vivo RCM. It provides the vertical view of ex vivo RCM and the horizontal view of in vivo RCM on melanocytic lesions. Therefore, more information on pigmented lesions can be gained, and more typical features of both techniques can lead the clinician to the correct diagnosis. In our study, we found that image quality was responsible for the diagnostic confidence level for LC-OCT and RCM. In general, reduced image quality is mainly associated with ulceration, crusting, or image taking experience. Nevertheless, it is not recommended to measure an ulcerated or crusted pigmented lesion with non-invasive devices, and in such cases a biopsy is needed. Clearly, the diagnostic confidence for each lesion depends not only on the quality of the LC-OCT image or the difficulty of the lesion, but also on the experience of the observer. Our clinicians were all similarly experienced, and therefore we did not evaluate observer variability. Hence, further studies with clinicians of different experiences with LC-OCT need to be performed. We also conclude that for the interpretation and analysis of LC-OCT images, tele-consulting might be useful for LC-OCT beginners and for discussing difficult cases. Furthermore, the diagnostic performance of LC-OCT for melanomas (vs. all nevi) has the same sensitivity and a better specificity compared to RCM. We only had two false negative cases with LC-OCT, where two melanomas were diagnosed as nevi. We reviewed the cases, and in the first case—a nevus-associated melanoma—we detected in the integrated dermoscopic view that only the dysplastic nevus part had been imaged. Thus, it is very relevant to ensure appropriate coverage of the whole lesion, or if the lesion is too big, that more images are taken. The second case—a big in situ superficial spreading melanoma—was of a bad image quality, more in LC-OCT (score 3) than in RCM (score 2), because of an air bubble due to technical issues at that time. In big-sized lesions, multiple measurements from different parts should have been taken. Due to the lesion's large size, a biopsy or surgery would have been performed anyway. Moreover, the diagnosis of a nevus vs. a dysplastic nevus is less accurate for both techniques. This aspect needs to be reconsidered, since one limitation of our study is that the number of dysplastic nevi in the subgroup of both devices was quite small ($n = 3$) vs. nevi ($n = 19$). A larger study for the evaluation of nevi vs. dysplastic nevi is required. One should keep in mind that histology can also be erroneous, especially in biopsies, because the entire lesion cannot be assessed here. Since the vast majority of the lesions were completely excised and serial sections including immunohistologies are standard in melanomas, we assume that this possible error is negligible.

Recently Lenoir et al. published a few LC-OCT criteria for benign dermal melanocytic proliferations such as wave pattern [28]. In our study, we evaluated the most significant criteria for diagnosing a melanoma vs. nevus with LC-OCT (in comparison with RCM). We found that an irregular honeycombed pattern, the presence of pagetoid spread, and the absence of dermal nests are the most important criteria to discriminate a melanoma from a nevus in LC-OCT. These findings are very similar to studies with RCM [29–31]. We were surprised that no criteria related to the DEJ could be detected here, but this can be explained by the fact that many superficial spreading melanomas have a well-defined DEJ, which were the majority of the melanomas in our study. In the future, a study about thin versus thick melanomas should be conducted, since Rudnicka et al. showed in RCM that there might be different key criteria [32].

5. Conclusions

In conclusion, our first study with the new LC-OCT device on melanocytic lesions showed that the discrimination between melanomas and nevi is possible. The improved distinction between nevi and melanomas with LC-OCT will lead to an immediate in vivo and non-invasive diagnosis, spare unnecessary surgeries if the lesion is diagnosed as a nevus, and therefore reduce health insurance costs and free capacities for other necessary melanoma surgeries.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/cancers14051140/s1>: Table S1: Most common LC-OCT parameters for melanocytic lesions; Table S2: LC-OCT key criteria more useful in discriminating a melanoma from a nevus (dysplastic or not); Table S3: Most common RCM parameters for melanocytic lesions; Table S4: RCM key criteria more useful in discriminating a melanoma from a nevus (dysplastic or not); Table S5: Analysis of the false negative cases.

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Data Availability Statement: Fully anonymized data are available on request.

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LETTER TO THE EDITOR

In vivo imaging of *Sarcoptes scabiei* infestation using line-field confocal optical coherence tomography

Dear Editor,

Scabies remains a worldwide spread disease of considerable health and economic burden with repeated outbreaks in community settings.¹ Scabies agent is the ectoparasite *Sarcoptes scabiei* var. *hominis*; this invisible mite causes a progressive rash, which can be misdiagnosed with eczema or *pruritus sine materia*. Diagnosis is based on characteristic clinical features (intense nightly itch,

inflammatory excoriated papules in typical sites) and history of contact to infected patients. According to the new International Alliance for the Control of Scabies (IACS) classification, the identification of mites, eggs or faecal pellets through skin scraping, high magnification devices or dermoscopy counts as diagnostic criteria for confirmed scabies.¹ The typical dermoscopic sign of scabies infestation is the so-called 'delta', 'kite' or 'triangle', representing the anterior body of the adult female mite at the end of the burrow² (Fig. 1e). Reflectance confocal microscopy (RCM)³ and optical coherence tomography (OCT)^{4,5} were used in the detection of mite infestations (scabies, demodex)^{6,7} and their therapeutic monitoring.^{6,7} Line-field confocal optical coherence tomography (LC-OCT, DAMAE Medical®, Paris, France) is new imaging technique that can also be used to visualize mites and

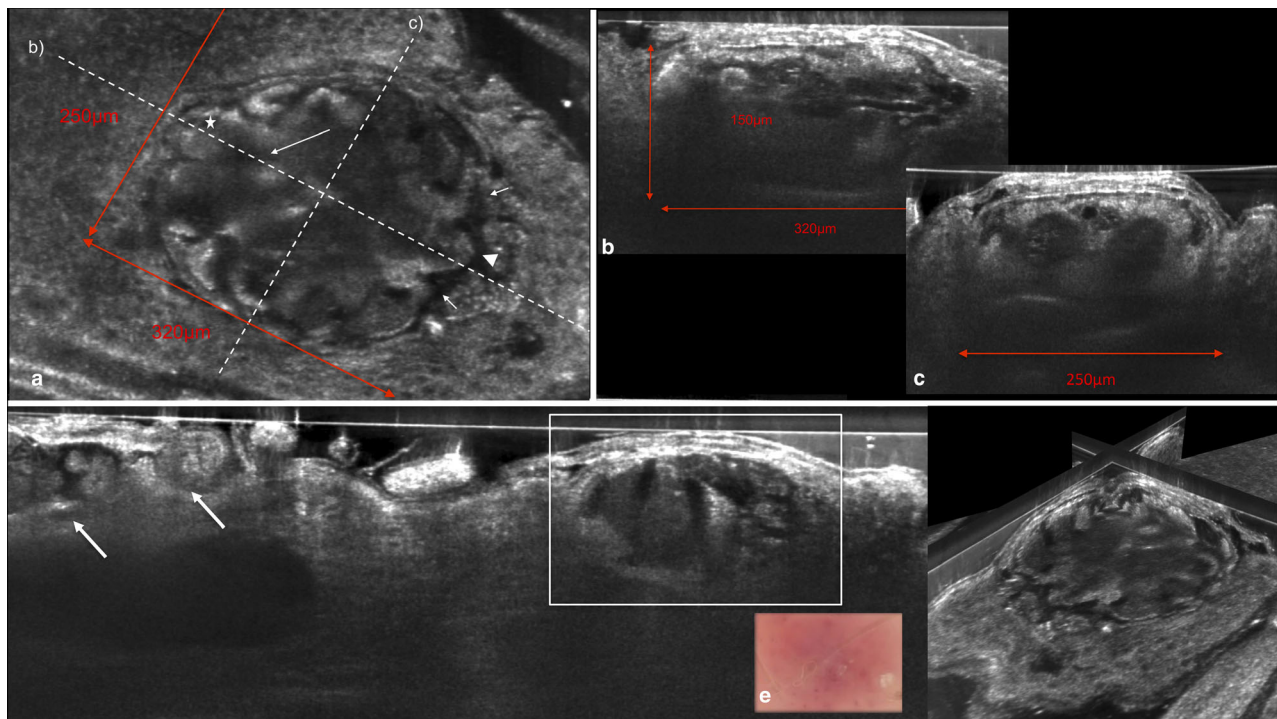


Figure 1 a) In en-face LC-OCT images, *S. scabiei* can be identified as a round structure in a burrow with a thin, corrugated exoskeleton and triangular spicules on the dorsal surface. Two pairs of hyperreflective coned structures (short arrows) corresponding to legs surround the polygonal head (triangle). At the opposite side, a triangular terminal anus (star) is visible and in the centre, a central, hyporeflective, convoluted gut (long arrow) with skybala and hyperreflective glands can be seen. 1b,c) Vertical LC-OCT images corresponding to the b) and c) axes in the 1A en-face image 1d) Vertical LC-OCT image with a thick hyperkeratotic burrow surrounding the mite inside the epidermis. The ovoid burrow behind the mite contains the hyperreflective skybala and eggs. 1e) Dermoscopy of the scabies mite with the so-called 'delta' or 'kite' or 'triangle' sign 1f) 3D reconstruction of the hyperreflective burrow containing the mite body.

related skin changes. LC-OCT is based on a two-beam interference microscope with line illumination and line detection using a broadband spatially coherent light source and a line-scan camera.⁸ It has three imaging modalities: en-coupe (vertical), en-face and 3D, providing a cellular resolution ($1.1 \times 1.3 \mu\text{m}$).

A patient (male, 50 years old) with suspect symptoms and skin rash was examined with LC-OCT (DAMAE Medical, Paris, France). Mites were also identified by high magnification digital dermoscopy (Fotofinder[®], Bad Birnbach, Germany). In LC-OCT en-face images, *S. scabiei* was clearly identifiable as an ovoid structure with a thin, corrugated exoskeleton and triangular spicules on the dorsal surface, localized right below the *stratum corneum* in a burrow (Fig. 1a). Two pairs of hyperreflective coned structures corresponding to legs surround the polygonal, even brighter head (Fig. 1a, triangle). At the caudal extremity, a triangular terminal anus is visible (Fig. 1a, star). Internal organs are also hinted at a central, hyporeflective, convoluted gut with skybala (Fig. 1, long arrow) and hyperreflective (probably ovary and vitelline) glands.

In LC-OCT vertical images (Fig. 1b,c,d) and 3D reconstruction (Fig. 1f), the exact location of the mite inside the epidermis and *Stratum corneum* can be observed. A thick hyperkeratotic scale corresponding to the burrow covers it. The optical density of the mite is comparable to the surrounding tissue; however, the hyporeflective burrow lumen and the scaly hyperreflective burrow wall build a fringe, which delimitates the mite body. An ovoid or longitudinal burrow can be also visualized behind the mite, depending on the scan direction. Skybala and eggs can also be identified as roundish, mainly hyperreflective structures.

The in vivo observation of scabies mites in their human hosts allows a quick diagnosis at bedside and provides interesting information about the biology of the living mites. Not only OCT and RCM but also LC-OCT permits fast, painless, real-time imaging of mites, their morphology and their skin location, with hints to their internal structures.^{3,9} LC-OCT has a better resolution of conventional OCT, but still lower than RCM; this is however compensated by the possibility of combining en-face and vertical views and obtaining 3D reconstructions at different angles. Burrows, eggs and skybala can also be visualized easily. The high resolution and penetration depth of LC-OCT combined with the possibility of acquiring vertical, en-face and 3D images provides more details compared to dermoscopy and other diagnostic techniques, which may possibly reduce diagnostic pitfalls. Moreover, exact special correlations and measurements are possible. This allows an immediate diagnosis of scabies infestation as well as non-invasive therapeutic monitoring.

We believe LC-OCT is a non-invasive diagnostic tool with high potential in dermatology, with a broad field of application

and likely to be applied in the future not only to skin cancer¹⁰ but also in skin infestations.

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Conflicts of interest

The authors declare no conflict of interest.

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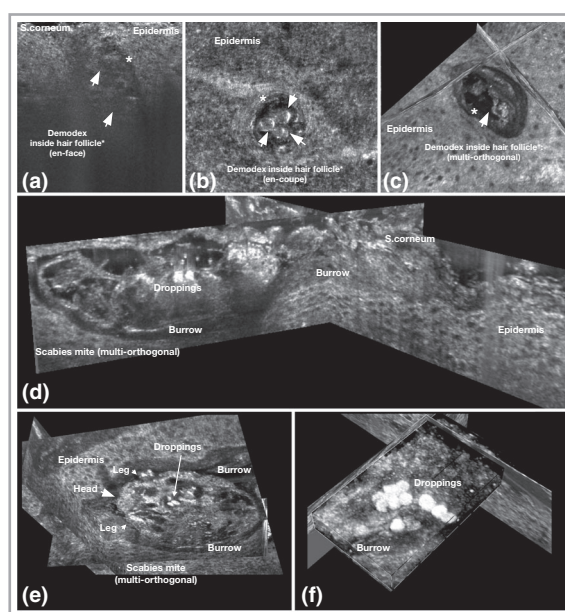
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Noninvasive real-time imaging of mite skin infestations with line-field confocal optical coherence tomography

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Dear Editor, Dermatologists treat several types of mites. Diagnosis is based on clinical features, dermoscopy and skin scraping. Besides reflectance confocal microscopy,¹ a new diagnostic imaging device, line-field confocal optical coherence tomography (LC-OCT),² provides real-time and bedside visualization of *Demodex* (a–c) and scabies mites (d–f) inside human skin. Multiview images show the locations in *en face*, vertical and multiorthogonal views. Observed features show *Demodex* bodies as round hyper-reflective structures inside the hair follicles (a–c) and scabies mite bodies as ovoid structures inside the dark burrows with hyper-reflective droppings (d–f). LC-OCT imaging allows quick diagnosis of cutaneous mite infestation and therapeutic follow-up.

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