Line-field confocal optical coherence tomography in melanocytic and non-melanocytic skin tumors

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ABSTRACT

INTRODUCTION: Line-field confocal optical coherence tomography (LC-OCT) is a recently introduced, non-invasive skin imaging technique combining the technical advantages of reflectance confocal microscopy and conventional OCT in terms of isotropic resolution and in-tissue penetration. Several studies have been published so far about the use of LC-OCT in melanocytic and non-melanocytic skin tumors. The aim of this review was to summarize the currently available data on the use of LC-OCT for benign and malignant melanocytic and non-melanocytic skin tumors.

EVIDENCE ACQUISITION: We searched scientific databases for any literature published up to 30th April 2023 and concerning the use of LC-OCT for melanocytic and non-melanocytic skin tumors. Identified papers were evaluated, and relevant information was extracted.

EVIDENCE SYNTHESIS: A total of 29 studies were found including original articles, short reports, and letters to the Editor: 6 applied to melanocytic skin tumors, 22 to non-melanocytic skin tumors and 1 to both. The use of LC-OCT increased the diagnostic accuracy for melanocytic and non-melanocytic skin lesions. The highest diagnostic performance was found for basal cell carcinoma (BCC), but significant improvements in the diagnostic accuracy were also detected for the differentiation of actinic keratosis (AK) from squamous cell carcinoma (SCC) and of melanoma from nevi. The LC-OCT features of other skin tumors were also described and successfully correlated with histopathology.

CONCLUSIONS: LC-OCT proved to increase the diagnostic accuracy for melanocytic and non-melanocytic skin lesions, thanks to the combination of high resolution/penetration, 3D reconstructions, and integrated dermoscopy. Although BCC seems the most suitable tumors for LC-OCT examination, the device is extremely performant for the differentiation of AK from SCC and the discrimination of melanoma from nevi as well. Additional studies on diagnostic performance and new investigations about the presurgical assessment of tumor margins with LC-OCT and its association with human and artificial intelligence algorithms are in progress.

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KEY WORDS: Tomography, optical coherence; Skin neoplasms; Carcinoma, basal cell; Carcinoma, squamous cell; Melanoma; Nevus.

Introduction

The most established non-invasive skin imaging techniques currently available on the market include reflectance confocal microscopy (RCM) and conventional optical coherence tomography (OCT). RCM provided images of the skin only in the horizontal plane (contrary to conventional histopathology) with a quasi-histological 1-µm lateral resolution and up to a 250-µm depth.[1] Conventional OCT provides a three-dimensional (3D) view of the skin (both vertical and horizontal planes), with bigger penetration depth (1-2 mm) but lower resolution (7.5 µm) than RCM.[2] Clearly then, these two techniques are complementary in terms of advantages and disadvantages.

Recently, a new technology has been developed, named line-field confocal OCT (LC-OCT; DAMAE Medical, Paris, France).[3] It provides vertical, horizontal as well as 3D reconstructions of the skin in real time, up to a 500-µm depth, and with excellent isotropic resolution (just above 1 µm) due to the high numeric aperture of the microscope objective (similar to that of RCM). Therefore, LC-OCT features a noteworthy combination of the advantages of conventional OCT (high penetration and visualization of the vertical plane) and RCM (high resolution). Interestingly, a dermoscopic camera has been recently integrated in the LC-OCT device, which allows a constant dermoscopic-imaging correspondence.

These unprecedented technical characteristics probably explain why LC-OCT has been gaining *momentum* in dermatological research. Over the last 5 years, numerous publications concerning this technology have seen the light of day, encompassing a wide spectrum of physiological and pathological dermatological conditions. In particular, the use of LC-OCT for different neoplastic, inflammatory, and infectious skin diseases have been reported.

The goal of the present study was to provide an up-to-date review of the literature concerning LC-OCT for benign and malignant melanocytic and non-melanocytic skin tumors.

Evidence acquisition

We searched various databases (PubMed/MEDLINE, ISI Web of Science, EMBASE, and Cochrane Library) for any literature published in English or French up to 30th April 2023 using the following keywords: "LC-OCT," "skin tumors," "melanocytic," "non-melanocytic," "melanoma," "nevi," "basal cell carcinoma," "squamous cell carcinoma," "actinic keratosis," "Kaposi sarcoma," "cutaneous vascular lesions," "extramammary Paget disease," "seborrheic keratosis," "sebaceous hyperplasia." No restriction of article type was applied (letters, clinical reports, and original papers were included). Identified articles were cross-referenced for articles missed by the database search.

Evidence synthesis

A total of 29 studies were found including original articles, short reports, and letters to the Editor:[3-31] 6 applied to melanocytic skin tumors,[4-9] 22 to non-melanocytic skin tumors,[10-31] and 1 to both[3] (Table I).[3-31]

LC-OCT in melanocytic skin tumors

The first ever LC-OCT images of a melanocytic lesion were published by Dubois *et al.*[3] In their introductory paper on the technology, the authors showed an example of cutaneous melanoma imaged by LC-OCT, in which one

TABLE I.—*Current evidence on LC-OCT for melanocytic and non-melanocytic skin tumors.*

Topic	Reference
Melanocytic skin tumors	
Benign dermal proliferations	Lenoir *et al.* (2021)[4]
Nevi and melanoma	Schuh *et al.* (2022)[5]
Nevi and melanoma	Perez-Anker *et al.* (2022)[6]
Lentigo maligna	Verzì *et al.* (2023)[7]
Agminated Spitz nevi	Del Río-Sancho *et al.* (2023)[8]
Pigmented lesions of genital area	El Zeinaty *et al.* (2023)[9]
Non-melanocytic skin tumors	
BCC	Suppa *et al.* (2021)[10]
	Ruini *et al.* (2021)[13]
	Verzì *et al.* (2021)[17]
	Cappilli *et al.* (2021)[14]
	Cappilli *et al.* (2022)[15]
	Cappilli *et al.* (2022)[16]
	Gust *et al.* (2022)[18]
	Cinotti *et al.* (2023)[19]
AK/SCC	Perrot *et al.* (2019)[21]
	Dejonckheere *et al.* (2019)[20]
	Lenoir *et al.* (2021)[22]
	Cinotti *et al.* (2021)[24]
	Ruini *et al.* (2021)[25]
	Ruini *et al.* (2021)[23]
	Fischman *et al.* (2022)[26]
	Cinotti *et al.* (2023)[27]
	Olesen *et al.* (2023)[28]
Vascular lesions	Tognetti *et al.* (2021)[29]
	Cappilli *et al.* (2023)[12]
Extramammary Paget disease	Di Stefani *et al.* (2023)[30]
Sebaceous hyperplasia	Lenoir *et al.* (2021)[11]
Seborrheic keratosis	Lenoir *et al.* (2023)[31]
Both melanocytic and non-melanocytic skin tumors	
Presentation paper on LC-OCT	Dubois *et al.* (2018)[3]

BCC: basal cell carcinoma; AK: actinic keratosis; SCC: squamous cell carcinoma.

could clearly appreciate the pagetoid spread (epidermal invasion) of epithelial tumor cells, the presence of intracorneal tumor cells in the process of being eliminated, the presence of dermal clusters of malignant melanocytes, the partial disruption of the dermal-epidermal junction (DEJ) and their histopathological correlates.

The first structured description of melanocytic lesions was subsequently provided by Lenoir *et al.*[4] The authors reported a series of 7 histopathologically confirmed benign dermal melanocytic proliferations (dermal and compound nevi) and suggested as the main LC-OCT criterion the so-called *wave-like* pattern. It consists in alternating undulated hyper-reflective and hypo-reflective lines in the papillary and reticular dermis, corresponding to the ridge of melanocytic strands/cords/nests (hyper-reflective) separated by spaces of variable thickness (hypo-reflective). These undulated structures are usually included in an ill-defined larger lobular structure, that correlates on histopathology with the presence of multiple melanocytic nests and can additionally contain bright roundish cells, corresponding to highly pigmented, benign melanocytes. Additional LC-OCT criteria for benign dermal melanocytic proliferations are: (i) well outlined DEJ (which can show hyper-reflective cells, isolated or in nests, in the case of compound nevi); (ii) upper papillary dermis thinned by the deeper melanocytic proliferation (visualized as a thin layer of homogenous hyper-reflective material immediately below the DEJ); and (iii) absence of atypical (large, dendritic, pleomorphic, hyper-reflective) cells in the epidermis/dermis, otherwise suggestive of an atypical melanocytic lesion.

The first study assessing the potential of LC-OCT in discriminating nevi from melanoma was performed by Schuh *et al.*[5] The authors analyzed 84 melanocytic lesions imaged with LC-OCT, of which 36 were also assessed by RCM. Importantly, they found that LC-OCT was not inferior to RCM when assessing melanocytic lesions: indeed, no difference in diagnostic performance between the two technology was detected for diagnosing a melanoma *versus* all types of nevi (RCM: 93% sensitivity, 95% specificity; LC-OCT: 93% sensitivity, 100% specificity). The study also suggested that the most significant criteria for diagnosing a melanoma with LC-OCT were irregular honeycombed pattern, pagetoid spread, and absence of dermal nests.

A subsequent study by Perez-Anker *et al.*[6] described the features of melanocytic lesions imaged by LC-OCT and their correlation with histopathology and RCM. In a series of 12 melanocytic tumors (2 *in situ* melanomas, 2 invasive melanomas, 4 atypical naevi, 2 intradermal naevi, 1 compound naevus and 1 junctional naevus), high correlation with 5-µm accuracy was detected between RCM and LC-OCT, at a cellular level. In particular, the study found striking similarities between the two techniques regarding all the most important diagnostic features (nests of melanocytic cells, ringed and meshwork pattern, and presence of dendritic and pagetoid cells). The ability of the RCM fixed probe to provide scans of the entire lesion *via* mosaicking remained as an advantage compared to the LC-OCT handheld probe. Of note, this is likely to be solved by next-generation LC-OCT devices soon to be introduced on the market and featuring a global dermoscopic colocalization of the LC-OCT acquisition within the entire surface of the lesion based on artificial intelligence (unpublished data). The authors concluded that LC-OCT allows the architectural and cellular characterization of different types of melanocytic lesions, with an excellent correlation with histopathology (vertical sections) and RCM (horizontal sections). Therefore, diagnostic criteria for melanocytic lesions already established with RCM can also be adopted with LC-OCT.

A recent case-report by Verzì *et al.*[7] focused on the LC-OCT features of lentigo maligna (LM), a subtype of *in situ* melanoma that classically presents in elderly patients as a slowly growing lesion on sun-exposed areas, that may evolve to invasive melanoma. In the case of a 49-year-old white man, the authors found that LC-OCT was able to detect *in vivo* the main microscopic features typical of LM, namely the presence of large, bright, roundish or dendritic atypical cells, with evident nuclei, corresponding to atypical melanocytes in the epidermis and around the hair follicles. A strong correspondence between vertical and horizontal LC-OCT images with vertical and horizontal histopathological sections was observed by the authors.

Another recent case-report by Del Río-Sancho *et al.*[8] focused on agminated Spitz nevi in a 6-year-old white girl. Vertical and horizontal LC-OCT examinations were able to non-invasively detect the presence of melanocytic nests adjacent to the DEJ and of melanophages in the papillary dermis as well as the absence of a pagetoid ascension of atypical melanocytes, with an excellent correlation with histopathology. The authors concluded that their case highlights the potential utility of LC-OCT for Spitzoid proliferations.

Most recently, El Zeinaty *et al.*[9] published a series of 9 patients with genital pigmented lesions (7 benign melanotic macules, 1 genital nevus, 1 invasive melanoma) imaged with LC-OCT. Divergent LC-OCT features were found in different lesions. Benign melanotic macules showed a thinned epidermis overlying a characteristically well-de-

fined, undulated and highly reflective chorion-epidermal junction (CEJ), abruptly transitioning to a flat non-reflective CEJ on the adjacent normal skin. The genital nevus showed a thinned epidermis overlying a well-defined, flattened, and pigmented CEJ characterized by bridging junctional nests of highly reflective melanocytes. The invasive melanoma showed a deranged architecture of the CEJ due to the high-density presence of atypical melanocytes in the form of bright dendritic cells at the basal layer and bright roundish cells in the basal and suprabasal layers (pagetoid proliferation).

LC-OCT in non-melanocytic skin tumors

Basal cell carcinoma

The first ever LC-OCT images of a basal cell carcinoma (BCC) were published by Dubois *et al.*[3] in their introductory paper on the technology. The presence of dermal nests of tumor cells (tumor islands) were clearly visible and correlated well with histopathology.

The first study investigating the use of LC-OCT in the field of BCC was subsequently performed by Suppa *et al.*[10] and included 89 histopathologically proven BCCs imaged with a LC-OCT device producing B-scans (vertically orientated sectional images). The study aimed at identifying/describing for the first time LC-OCT criteria associated with BCC as well as at exploring their association with BCC subtypes. The most frequent LC-OCT criteria for BCC were dermal lobules and blood vessels, which incidentally represent the most useful diagnostic criteria in histopathology and, respectively, dermoscopy. In particular, lobules represent the most important LC-OCT criterion for BCC recognition, similarly to what happens in histopathology. Upon LC-OCT examination, lobules are characterized by three components: 1) a grey core (laminated structure orientated along the horizontal plane; named "*millefeuille* pattern", as it resembles the pattern seen in the eponymous French delicacy *millefeuille*; corresponding to the dense cellularity within the basaloid tumor island, composed of basaloid cells, immune cells, apoptotic bodies, and mitotic figures); 2) middle dark rim (immediately surrounding the core of the lobule; named "clefting;" corresponding to peritumoral mucin deposition); 3) outer bright rim (surrounding the lobule and characterized by a brighter color than the stroma; corresponding to the compression/alteration of the collagen fibers of the stroma by the tumor island, due to mass effect and tumor-stroma interaction). The simultaneous presence of inner lamination, middle clefting and outer bright rim gives rise to a triad of colors (grey, black, and white, respectively). Importantly, the description of the *millefeuille* pattern is of clinical relevance. Such level of detail in the characterization of the core of the BCC lobule (possible thanks to the unprecedented technical characteristics of LC-OCT in terms of high resolution, high penetration, and vertical/3D visualization) can facilitate the differential diagnosis of BCC from other entities characterized by the presence of dermal lobules, such as sebaceous hyperplasia (lobules characterized by the so-called *granular-lobular* pattern; Figure 1),[11] dermal nevus (lobules characterized by the so-called *wave-like* pattern),[4] melanoma (lobules characterized by atypical melanocytic cells),[6] and cutaneous vascular lesions (lobules characterized by dark vascular lacunae; Figure 2).[12] Interestingly, the lobule shape, size, and location were independent predictors of BCC subtype: hemispheric lobules connected to the epidermis were associated with superficial BCC (sBCC); macrolobules separated from the epidermis with nodular BCC (nBCC); branched lobules with infiltrative BCC (iBCC). Prototypic criteria of each BCC subtype can be generally observed simultaneously in mixed subtypes, *e.g.* coexistence of hemispheric and branched lobules in superficial and infiltrative BCC (siBCC).

In a parallel investigation, Ruini *et al.*[13] evaluated 52 histopathologically confirmed BCCs and similarly found that LC-OCT allows the non-invasive, real-time BCC

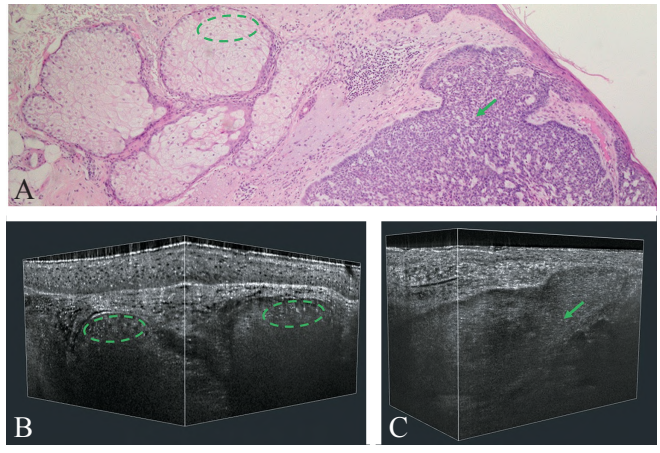

Figure 1.—Comparison of lobules of sebaceous glands and basal cell carcinoma on histopathology (A) and LC-OCT (B, C). Upon LC-OCT examination, lobules of sebaceous glands (B) are characterized by a granular-lobular pattern at the top of the lobule, corresponding to sebocytes on histopathology (green circle), and by a loss of signal at the bottom of the lobule; C) lobules of basal cell carcinoma are characterized by a millefeuille pattern covering the entirety of the lobule and corresponding to the dense cellularity of the basaloid tumor islands on histopathology (green arrow).

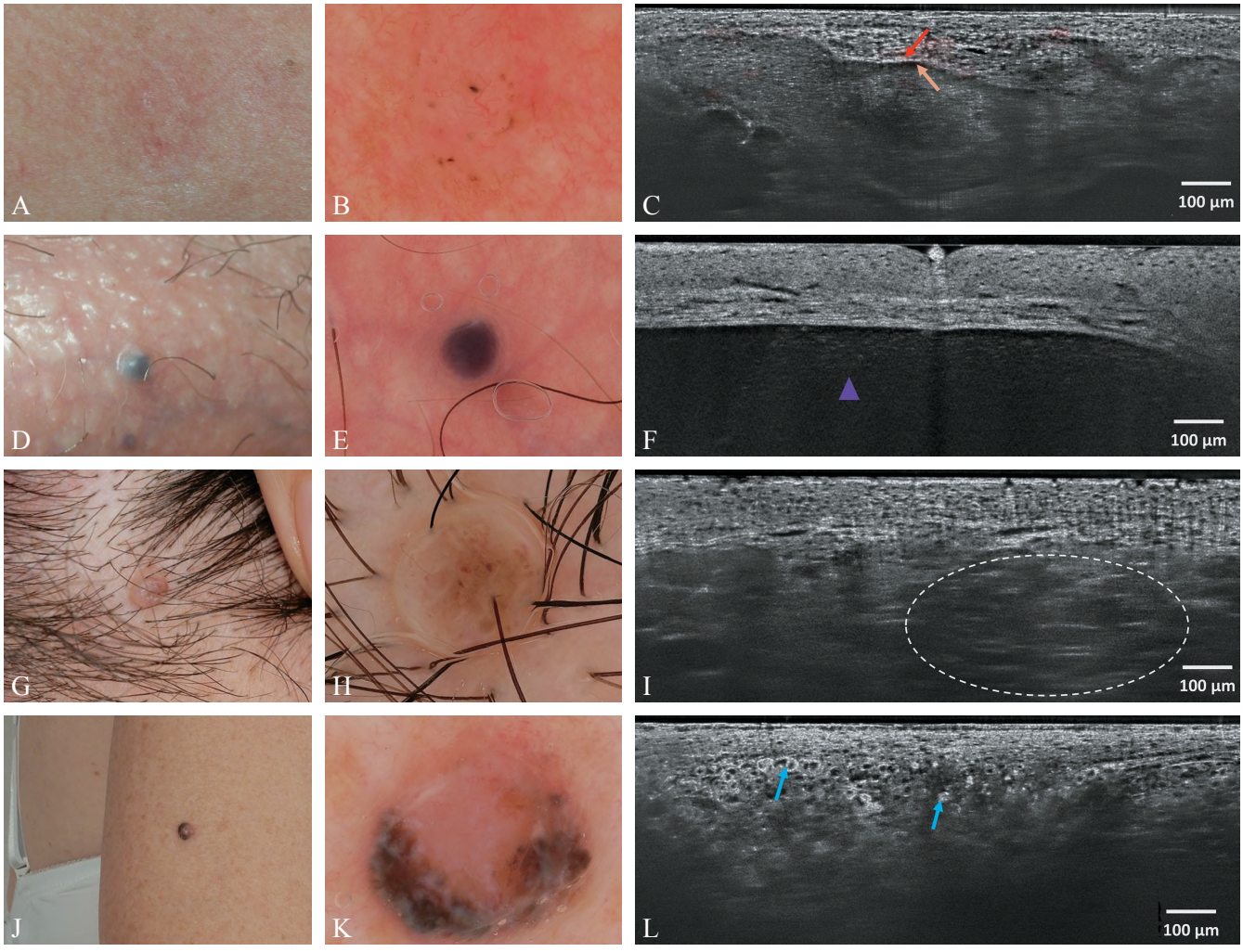

Figure 2.—Clinical, dermoscopic, and vertical LC-OCT presentation of nodular BCC (A-C), hemangioma (D-F), dermal nevus (G-I), and nodular melanoma (J-L). Upon LC-OCT examination, the core of the dermal lobule is characterized by a *millefeuille* pattern delimited by its clefting (orange arrow) and bright rim (red arrow) in the nodular BCC (C); by a homogeneous dark vascular lacuna (purple triangle) in the hemangioma (F); by a wave-like pattern (white circle) in the dermal nevus (I); and by tightly packed bright pleomorphic cells with dark nuclei and bright cytoplasm (blue arrows) corresponding to atypical melanocytes in the nodular melanoma (L).

recognition and subtyping in vertical, horizontal, and 3D mode compared with histopathology, RCM, and conventional OCT. The most useful LC-OCT criteria for BCC subtyping were: 1) for sBCC, the thickening of the epidermis due to a series of tumor lobules with clear connection to the DEJ (*string of pearls* pattern); 2) for nBCC, tumor nests in the dermis with dark clefting and prominent perilobular vascularization, as well as atypical epidermal keratinocytes, altered DEJ, and white hyper-reflective stroma; 3) for iBCC, elongated hyporeflective tumor strands, surrounded by bright collagen (*shoal of fish* pattern). Interestingly, the authors found an overall BCC subtype agreement rate of 90.4% between LC-OCT and conventional histopathology, suggesting that LC-OCT can be regarded as a useful tool to correctly subtyping BCC without the need of a biopsy.

Of course, the complete examination of the lesion with the LC-OCT probe is paramount to ensure the correct subtype classification of BCC. A case illustrating the importance of LC-OCT full-lesion examination was reported by Cappilli *et al.*[14] The authors presented the case of a mixed-subtype BCC (siBCC) erroneously classified as sBCC because it was imaged only on the superficial component, leaving the infiltrative component (located at the periphery

of the lesion) out of the examination. To avoid such misdiagnoses, it is important to use either adhesive paper reinforcement rings applied to different portions of the lesion or the integrated dermoscopic camera (made available in a more recent, new-generation LC-OCT device) by which the examiner constantly knows which portion of the lesion is being imaged, through an exact dermoscopic-imaging correspondence.

The LC-OCT characterization of additional BCC subtypes was recently reported by our group. Cappilli *et al.*[15] identified and described the characteristics of metatypical BCC or basosquamous carcinoma (BSC), a rare and potentially aggressive cutaneous neoplasm combining histopathological features BCC and squamous cell carcinoma (SCC). After the analysis of a series of 6 BSC, the authors concluded that LC-OCT may support the non-invasive recognition of BSC through the simultaneous detection of BCC-associated (dermal lobules with *millefeuille* pattern, dilated vessels, bright cells within the epidermis, bright cells within lobules, stromal stretching, stromal brightness) and SCC-associated features (acanthosis, hyperkeratosis, disarranged epidermal architecture, broad strands, elastosis, and glomerular vessels, as reported below). Interruption of the DEJ and ulceration represented overlapping criteria.

Later, Cappilli *et al.*[16] identified and described the characteristics of fibroepithelioma of Pinkus (FeP), a rare cutaneous tumor traditionally considered as a low-risk variant of BCC. Their analysis of a series of 5 cases indicated that LC-OCT can support the non-invasive diagnosis of FeP through the detection of the so-called *fenestrated* pattern, corresponding to that described in histopathology and featuring branched lobules (originating from the epidermal basal layer; anastomosing downward in the dermis; exhibiting the typical millefeuille pattern, clefting and peripheral bright rim; corresponding to the FeP strands) and overwhelming stroma (stretched, bright and outlined by the FeP strands).

A pilot study by Verzì *et al.*[17] suggested that LC-OCT could also be regarded as a promising tool to evaluate the treatment response of BCC to non-invasive treatments. Indeed, out of a series of 20 sBCCs treated with the immune response modifier imiquimod 5% cream (once daily, five days a week, for six weeks), the authors were able to rapidly and non-invasively detect 3 cases of residual BCC that would have gone otherwise undetected at clinical/dermoscopic examination.

Currently, two studies on the diagnostic performance of LC-OCT in the field of BCC are available. In the first study, Gust *et al.*[18] investigated the accuracy of LC-OCT in correctly diagnosing and classifying clinically equivocal cases of BCC compared to dermoscopy alone in a retrospective evaluation of 182 lesions prospectively enrolled from 154 patients. Diagnostic confidence was rated as "high" in only 48% of the study lesions with dermoscopy alone compared to 70% with LC-OCT. Overall, the authors found a 3%-increase in diagnostic accuracy from dermoscopy [88% (sensitivity 90%; specificity 86%)] to LC-OCT [91% (sensitivity 98%; specificity 80%)]. However, when considering the subgroup of LC-OCT images with a high diagnostic confidence, the increase in diagnostic accuracy of LC-OCT compared to dermoscopy was of 10.5% [98.5% (sensitivity 100%; specificity 97%)]. As for distinguishing between superficial BCCs and other BCC subtypes, LC-OCT had 90%-accuracy (sensitivity 77%; specificity 96%).

In the second study, Cinotti *et al.*[19] calculated the diagnostic performance of LC-OCT in a real-life investigation including 243 benign and malignant skin lesions consecutively enrolled within a 13-month time span. Albeit no change in sensitivity, they found a significant increase in specificity with the use of LC-OCT compared to dermoscopy alone, mainly driven by the ability of LC-OCT to differentiate BCCs from other diagnoses (specificity: 86% for dermoscopy, 95% for LC-OCT).

Even better parameters of diagnostic performance are to be expected from three soon-to-be-published studies carried out by our group, one retrospective (Mtimet *et al.*, manuscript submitted), one prospective (Orte Cano *et al.*, unpublished data) and one based on the retrospective evaluation of prospectively enrolled lesions by means of a diagnostic algorithm (Aktas *et al.*, unpublished data).

Actinic keratosis and squamous cell carcinoma

The first notions of LC-OCT for actinic keratosis (AK) and SCC in the literature could be found in 2019. Back then, Dejonckheere *et al.*[20] suggested for the first time the potential of LC-OCT as an important diagnostic approach in the cancerization field (actinic dysplasia syndrome) and published the first ever 2D *in-vivo* LC-OCT images of an AK and a SCC.

Similarly, Perrot *et al.*[21] published the first ever 3D *ex-vivo* LC-OCT image of a SCC in the shape of a 1200 µm x 1200 µm x 500 µm cube, showing the potential of the device in illustrating the main characteristics of this skin tumor in comparison with the adjacent healthy skin.

The first studies reporting LC-OCT criteria for keratinocyte skin tumors [AK, Bowen's disease (BD), SCC] were

published in 2021. Lenoir *et al.*[22] reported the main morphologic features observed in AK on LC-OCT, supported by 16 histopathologically confirmed cases: 1) hyperkeratosis (variable, irregular, hyperreflective); 2) pleomorphism (cellular and nuclear, in the basal and upper layers of the epidermis); 3) well outlined DEJ along the entire lesion (which is likely to exclude the possibility of an invasive SCC). These criteria well correlated with histopathology. Moreover, the different histopathological patterns could also be observed upon LC-OCT examination: atrophic epidermis without its rete ridges (in atrophic AKs); hypertrophic epidermis with thicker rete ridges (in hypertrophic AKs); basal layer showing broad-based buds (tumor budding) towards the papillary dermis (in proliferative AKs); discohesive bundles of acantholytic keratinocytes within the epidermis (in acantholytic AK). The authors also discussed the intrinsic limitations of LC-OCT in the field of AK/SCC, *i.e.* its limited ability to examine highly hyperkeratotic and acanthotic lesions: indeed, hyperkeratosis may cause excessive hyper-reflectivity of the upper part of the lesions and reduce the image quality of the underlying skin layers; and acanthosis may render impossible for the LC-OCT examination to reach the DEJ thus hampering its visualization.

The ability of LC-OCT in visualizing the basal growth pattern of AK was further investigated by Ruini *et al.*[23] In a pilot study including 50 histopathologically proven AKs, the authors demonstrated that LC-OCT is able to reproduce *in vivo*, with strong interobserver agreement and a good correlation with histopathology, the so-called PRO classification, a model focused on different stages of downward extension of basal keratinocytes, "protruding" into the papillary dermis (PRO I, "crowding" of atypical keratinocytes in the basal layer; PRO II, "budding" of atypical keratinocytes in round nests into the upper papillary dermis; PRO III "papillary sprouting" of atypical keratinocytes protruding into the dermis and thicker than the overlying epidermis).[32-34] Thus, the authors suggested that LC-OCT can non-invasively classify AKs based on the basal growth pattern of keratinocytes and, therefore, their progression risk into invasive SCC.

Two studies published simultaneously assessed for the first time whether LC-OCT is able to discriminate between AK and SCC. Cinotti *et al.*[24] carried out a study on 158 lesions including 50 AKs and 108 SCCs in order to identify/describe LC-OCT criteria associated with SCC and AK, and to compare LC-OCT findings in these tumors. They found that cytological/architectural alterations such as epidermal pleomorphism, cellular atypia, and dilated/glomerular vessels were found in both AK and SCC (*in situ* and invasive), whereas an outlined DEJ without broad strands was observed in almost all AKs and almost all *in situ* SCCs, but almost never in invasive SCC when the DEJ was detectable (again, the visualization of the DEJ was often hampered by hyperkeratosis and/or acanthosis). These data suggested that LC-OCT can help clinicians in the identification of AK and SCC and their differentiation, providing a real-time and non-invasive examination.

Similar conclusions were found by Ruini *et al.*,[25] who performed a study on 73 histopathologically confirmed lesions aimed at defining the main imaging criteria and histological correlates of AK, BD and SCC using LC-OCT. The authors found that all types of keratinocyte cancers displayed variable degrees of hyperkeratosis/parakeratosis, disruption of stratum corneum, broadened epidermis, basal and suprabasal keratinocyte atypia, dilated vessels/neoangiogenesis and elastosis/collagen alterations. Moreover, AK and BD were associated with a preserved DEJ, Bowen's disease with marked keratinocyte atypia involving all epidermal layers (bowenoid pattern), and SCC with ulceration, increased epidermal thickness, keratin plugs, acantholysis, not visible/interrupted DEJ and epidermal bright particles. Importantly, LC-OCT increased the diagnostic confidence by 24.7% compared with dermoscopy alone.

Subsequently, an important study performed by Fischman *et al.*[26] attempted to overcome the subjectivity of the notion of cellular atypia/dysplasia of AK/SCC and within the field of cancerization, which classically suffers poor inter-rater agreement in the scientific literature. The authors developed a deep learning algorithm trained to segment keratinocyte nuclei from 3D LC-OCT images and derived quantitative, reproducible, and biologically relevant metrics that enabled objective definitions of keratinocyte atypia. Therefore, they demonstrated that the notion of cellular atypia can be objectively defined and quantified with a non-invasive, *in-vivo* approach in 3D. This has huge clinical relevance, as it could standardize severity assessment and treatment monitoring in the field of AK and SCC.

Recently, Cinotti *et al.*[27] compared for the first time LC-OCT and RCM for the identification of the different features of keratinocyte skin tumors (AK and SCC). They performed a multicentric study including 52 lesions (33 AKs and 19 SCCs) and found that, while irregular epidermis and erosion/ulceration were equally visible with both techniques, LC-OCT was significantly superior to RCM in visualizing parakeratosis, dyskeratotic keratinocytes, linear dilated and glomerular vessels.

In an attempt to demonstrate the potential of ablative fractional laser as a prevention strategy for SCC and photodamage in high-risk populations, Olesen *et al.*[28] recently found that repeated ablative fractional laser treatments of sun-exposed skin in a preclinical UVR-induced SCC mice model significantly delayed SCC tumor formation and prevented signs of photodamage assessed both clinically and with LC-OCT. In particular, LC-OCT was successfully used for the assessment of subclinical photo-prevention based on normalization of keratinocyte dysplasia, dermo-fiber morphology (collagen and elastin fibers), and skin thickness.

Kaposi sarcoma and other cutaneous vascular lesions

Tognetti *et al.*[29] reported the first case of Kaposi sarcoma imaged with LC-OCT on the glans of a 67-year-old man. LC-OCT showed the presence of abundant dermal vessels visible as dark areas separated by solid structures: short dark areas (corresponding to slit-like vessels) were visible in the papillary dermis; long/large dark areas (corresponding to vascular lacunae) were detected in the deeper dermis; small roundish dark areas (corresponding to dilated capillary loops in the dermal papillae, *i.e.* dotted vessels on dermoscopy) were found at the periphery of the lesion. The authors concluded that the ability of LC-OCT in detecting the aberrant vascular proliferation and the solid tissue among vascular channel typical of Kaposi sarcoma may help in distinguishing it from other benign vascular proliferations.

Indeed, in a recent study focusing on cutaneous vascular lesions imaged by LC-OCT, Cappilli *et al.*[12] found that the *in-vivo* detection of an increased dermal vascularity with different morphology may provide practical clues for the differential diagnosis of different vascular lesions, including Kaposi sarcoma. The investigation was based on 71 histopathologically proven vascular lesions, including 25 cherry hemangiomas, 15 angiokeratomas, 10 thrombosed hemangiomas, 6 pyogenic granulomas, 5 venous lakes, 4 targetoid hemosiderotic hemangiomas, 4 Kaposi sarcomas, and 2 glomuvenous malformations. In all study lesions, LC-OCT detected increased dermal vascularity, assuming different size/shape according to the particular type of vascular lesion and with good correlation with histopathology.

Extramammary Paget disease

Di Stefani *et al.*[30] recently reported the case of an extramammary Paget disease (a rare cutaneous adenocarcinoma arising in apocrine gland-rich regions such as genital and axillary areas) imaged with LC-OCT. Nests and solitary cells, larger than normal keratinocytes, were observed in the epidermis and at the DEJ both in vertical and horizontal LC-OCT images, corresponding to atypical epithelial cells with abundant clear cytoplasm and pleomorphic, hyperchromatic nucleus in histopathology following a confirmatory biopsy. Additionally, the authors also made use of LC-OCT to design the lesion margins prior to radiotherapy.

Sebaceous hyperplasia

Lenoir *et al.*[11] reported the LC-OCT features of sebaceous hyperplasia (one of the most frequently encountered lesions in dermatological practice, but that can be sometimes mistaken for a nBCC) based on a series of 12 histopathologically confirmed cases. The authors described the so-called *granular-lobular* pattern (well-defined roundish lobular formations in the dermis, containing hypo/hyper-reflective granular structures of variable size, corresponding to the cytoplasmic lipid droplets and nuclei of sebocytes) as the main criterion of sebaceous hyperplasia, in contrast with the previously described *millefeuille* pattern typical of BCC.[10] Another important criterion was the presence of connection between the lobular structures and a hair follicle, also important for the differential diagnosis with BCC and best observed upon the 3D LC-OCT reconstruction.

Seborrheic keratosis

More recently, Lenoir *et al.*[31] reported the LC-OCT features of seborrheic keratosis (SK) based on a series of 29 histopathologically proven cases: 1) acanthotic proliferation of non-pleomorphic keratinocytes in the epidermis; 2) presence of pseudo-horn cysts in the shape of roundish hyperreflective structures in the epidermis; and 3) extension of the rete ridges in the dermis, limited by a straight horizontal line, similar to the 'string sign' described in histopathology. Furthermore, the authors suggested that LC-OCT can detect different architectural patterns of SK, which correspond to different histopathological subtypes: limited acanthosis (flat SK); prominent acanthosis (acanthotic SK); large papillomatous epidermal expansions with irregular thickening of the stratum corneum (hyperkeratotic SK); hyperreflective keratinocytes proliferating in a reticular pattern at the basal layer (pigmented reticulated SK) or in full-thickness in an acanthotic epidermis (melanoacanthoma).

Conclusions

In conclusion, 29 studies have been published so far on the subject of LC-OCT for melanocytic and non-melano-

cytic skin tumors. When in the hands of expert clinicians, the device proved to increase the diagnostic accuracy in both fields, thanks to its unprecedented combination of high resolution, high in-tissue penetration, rapid 3D skin reconstructions, and integrated simultaneous dermoscopy. Among the examined skin tumors, BCC seems to be the most suitable one for LC-OCT examination as the diagnostic performance of the device for this neoplasm nears that of the gold standard, histopathology. However, LC-OCT also proved extremely performant for the differentiation of AK from SCC and the discrimination of melanoma from nevi. Additional studies are needed to make the evidence on the diagnostic performance of LC-OCT even more robust and to explore new, interesting applications such as the use of LC-OCT in the presurgical assessment of tumor margins and its association with human and artificial intelligence algorithms. The era of LC-OCT in the management of melanocytic and non-melanocytic skin tumors has just started.

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Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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