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Reflectance Confocal Microscopy in Melanoma and Non-melanoma Skin Cancer

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Abstract

Reflectance confocal microscopy (RCM) is a new technique enabling the visualization of the skin at a quasi-histological resolution, allowing the identification of clues for the diagnosis of skin diseases. The aim of this analysis was to provide new insights into the role of RCM in the diagnosis of skin cancers. Data comes from the most recent literature, taking into account previous essential reported information in this field. The study eligibility criteria were: studies providing update information, focusing on RCM findings in melanoma and non-melanoma skin cancers (NMSC), without restrictions for age, sex, ethnicity. Duplicated studies and single case report were excluded from this study. A search concerning the role of RCM in melanoma and NMSC was performed on the Medline. RCM clues were analyzed for different skin cancers, in particular melanoma and NMSC, in association with clinical, dermoscopic and histopathologic findings. Diagnostic accuracy, sensibility and specificity of the technique were reviewed. Furthermore, some new findings have been described and recent applications have been discussed. The selection of articles was limited in order to provide an up-todate revision. In conclusion, several RCM features were implemented for the diagnosis of melanoma and NMSC, leading to a confocal-based classification in most cases.

Keywords: Microscopy, confocal - Skin neoplasms - Melanoma.

Introduction

Reflectance confocal microscopy (RCM) is one of the latest tools introduced in non-invasive imaging in dermatology. This technique enables in vivo observation at a quasi-histological resolution of the epidermis, dermoepidermal junction (DEJ) and upper dermis, providing horizontal grayscale color images related to refractive index of different tissues and cell structures (Calzavara-Pinton et al. 2008, 1421–30). The highest refractivity is shown by melanin, contained in melanosomes, melanocytes, melanophages and pigmented keratinocytes, followed by structures containing keratin (Rajadhyaksha et al. 1995, 946–52). Noteworthy, cellular contrast in tissue can also be obtained when melanin is present in very small quantities, thanks also to the brightness of some subcellular organelles or surrounding structures (Rajadhyaksha et al. 1999, 293–303). Therefore, RCM can be used for the evaluation of both melanocytic and nonmelanocytic skin lesions.

Several criteria have been described in order to improve diagnostic accuracy (Pellacani, Cesinaro and Seidenari 2005, 979–85). Furthermore, the maximum depth achieved by RCM is approximately 250 µm,3 thus impairing the visualization of the deepest part of skin (Segura et al. 2008, 1311–20; Longo et al. 2013, 58–67). With increasing knowledge, RCM is giving more dynamic insights in the diagnosis of skin cancers.

In order to provide an updated review on the application of RCM, data coming from the most recent literature will be reported and critically revised. A focus on diagnostic accuracy and diagnostic criteria for malignant melanomas and non-melanoma skin cancers (NMSCs) will be provided.

RCM updates for examination of skin cancers

Basic principles of RCM

RCM is a non-invasive method for high-resolution diagnostics of tissue. While conventional microscopes work by using transmitted light technology with thin tissue layers being illuminated from below, RCM works with incident light technology. The skin is illuminated from above with a focused laser thus the reflected light is directed through a pinhole onto a detector so that only signals from a defined horizontal plane are used for high-resolution imaging allows the penetration depth into the skin. This non-invasive technique permits *in vivo* and in real time examinations, allowing microscopic images of skin layers close to the surface and opens up new possibilities for dermatology to monitor both diagnose and progression, in particular in the cases of dynamic changes. It also can be used ex vivo with freshly excised tissue, resembling a frozen section analysis, which is interesting especially for the field of microscopically controlled surgery of skin tumors.

RCM uses a diode laser as a source of monochromatic light that penetrates into the skin and illuminated a small points inside the tissue. The light is reflected, goes through a small pinhole and forms an image in the detector. This small pinhole allows only reflected light from the focal region (confocal) to reach the detector, rejecting all the coming light from out-of-focus plane (Kolm and Braun 2012, 7–10). The reflected light signals from a horizontal plane is then detected, where the refractive index changes. Structures with a higher refractive index appears bright in RCM, such as keratin, melanin and collagen, that result white in RCM.

Notes on the imaging technique

Examinations of a skin lesion is easy. A polymer or glass window is attached to a metal ring ad fixed to the skin with a special adhesive tape to reduce skin movement, where a small amount of immersion oil is applied to the skin. Afterwards, a water-based gel is applied inside the ring. This immersion liquid have refractive index close to that of the epidermis (1.34), thus reducing the spherical aberration of the beam passing through air and therefore allowing sufficient imaging through the epidermis and into the dermis The ring is then magnetically connected to the objective lens housing to stabilize the site of imaging (Kolm and Braun 2012, 7–10; Wurm, Kolm and Ahlgrimm-Siess 2012, 11–19).

RCM images are obtained horizontally from the lesion. Each single image displays a 500µM x 500µM large field-of-view on the screen. It is therefore possible to scan the entire area of the lesion up to 8mm x 8mm. In this way, it is generated a mosaic grid of contiguous horizontal images. This two-diensional composite mosaic is called *VivaBlock*. Additionally, an automated vertical sequence of images, each 500µM field-of-view, can be captured in depth, providing a three-dimensional view of certain area. This is called *VivaStack*. The distance between each section and the section depth can be adjusted. Another useful function is recording a video at 15-20 frames per second, to document dynamic events such as blood flow, or migration of leucocytes. For easier correlation of the macroscopic image and the confocal image, a digital macro camera (Vivacam; Lucid Inc) is linked to the RCM. This camera produces a 5 mega-pixel dermoscopic image of a 10mm field of interest enabling direct viewing of the dermoscopic structures on the RCM monitor. It is possible to navigate within the dermoscopic image and to specify areas for the subsequent RCM viewing and therefore allows choosing the interesting areas in this larger lesion and better asses the borders of a lesion which can be very helpful for surgical planning (Kolm and Braun 2012, 7–10).

RCM acquisition can be carried out with different available tools. The introduction of the handheld RCM device, Vivascope 3000® (Caliber Imaging and Diagnostics, Rochester, NY, USA), permits a better access to difficult anatomic locations and can be used for clinically and dermoscopically equivocal lesions in areas including nasal region, scalp, eyelids, ears and mucosae in order to differentiate benign lesions from skin cancer or for a rapid determination of the nature of the lesion for example melanocytic versus nonmelanocytic (Castro et al. 2015, 1164–69; Fraga-Braghiroli et al. 2014, 933–42; Cinotti et al. 2014, 912–918.e2; García-Hernández et al. 2013, 205256). Clinical applications include the evaluation of recurrences,

assessment of margins for excision like for lentigo maligna (LM)/lentigo maligna melanoma (LMM) and basal cell carcinoma (BCC) and monitoring of non-invasive treatments for AKs (Champin et al. 2014, 247–56; Guitera et al. 2013, 692–98; Longo et al. 2014, 716–724.e1; Ulrich et al. 2010, 15–24).

On the other hand, the wide-probe (Vivascope 1500®, MAVIG GmbH, Munich, Germany) allows a broad non-invasive examination of the epidermis and papillary dermis at cellular-level resolution viewing mosaics (Vivablock®), with a maximum size of 8x8 mm, at epidermis, DEJ and upper dermis level. This probe is the most appropriate for differential diagnosis between nevus and melanoma, because the visualization of the entire lesion is possible and the Vivacam® (Caliber Imaging and Diagnostics, Rochester, NY, USA) (Longo et al. 2015, 31–41) guarantees an overlap with dermoscopic and, recently, also clinical features.

RCM provides substrates for dermoscopic patterns, corresponding to specific histopathological criteria (Pellacani et al. 2008, 1597–1608). This means that, in specific cases, RCM can avoid a skin biopsy while possibly reducing patientrelated costs for excisions. Furthermore, RCM allows the non-invasive exploration of the entire lesion, similarly to what obtained with a shaving biopsy (Wolberink et al. 2013, 985–89).

Diagnostic accuracy and number needed to excise

While histopathology remains the gold standard for the diagnosis of skin cancers, dermoscopy and RCM analysis can improve an early recognition of these lesions.6 Previous reports described the high sensibility and the increased specificity supporting the analysis of equivocal melanocytic and nonmelanocytic lesions (Guitera et al. 2012, 2386–94; Lovatto et al. 2015, 1918–25), with a better differentiation among skin cancer simulators and interpretation of clinical and pathological correlation (Larre Borges et al. 2014, 833–45; Longo et al. 2012, 799–814; Pellacani et al. 2014, 864–72; Longo et al. 2013, 125–31;). It has been proved that systematic application of RCM diagnostic algorithms showed a specificity ranging between 50% and 70% when evaluating equivocal lesions (Guitera et al. 2012, 2386–94; Pellacani et al. 2014, 1044–51).

Interestingly, differences in diagnostic accuracy between expert and recent RCM users have been reported (Farnetani et al. 2015, 1075–80), considering that formal training programs have been recently introduced. The mean sensitivity was 88.9% while the mean specificity was 79.3%; experienced RCM users showed higher sensitivity compared with recent users.

Another important issue addressed in the last few years regarded the number needed to excise (NNE). A reduced number of benign lesions excised associated with an improved diagnostic accuracy were obtained with RCM evaluation of lesions eligible for surgical excision because dermoscopically suspected to be malignant or showing dermoscopic changes during the follow-up (Pellacani et al. 2014, 1044–51; Ferrari et al. 2015, 1135–40; Alarcon et al. 2014, 802–8; Stanganelli et al. 2015, 365–71). In particular, in a cohort study enrolling 1005 patients, 423 of which presenting lesions suspected to be malignant, RCM examination reduced the number of lesions for excision to less than one half of benign lesions, passing from a NNE ranging from 8.7 to 29.4 with dermoscopy to 6.8 after RCM evaluation (Pellacani et al. 2014, 1044–51). A previous study (Alarcon et al. 2014, 802–8), performed in a different setting, reported a lower NNE from 3.73 to 2.87.

Dysplastic nevi

Dysplastic nevi represent major risk factors for melanoma and they are challenging lesions both clinically and histopathologically (Elder 2010, 112–20; Duffy and Grossman 2012, e1-16). A simplified algorithm was developed by Pellacani et al. (Pellacani et al. 2012, e109-121) in order to differentiate nevi, dysplastic and nondysplastic, from melanomas. 60 lesions among which 19 nondysplastic nevi, 27 dysplastic nevi and 14 melanomas were analyzed by RCM and histopathology.

RCM criteria found in melanocytic lesions are presented in Table I.

Upon RCM, dysplastic nevi were usually characterized by a ringed and meshwork patterns with irregular junctional nests showing short interconnections or atypical junctional cells mostly located in the center of the lesion. In de-

tails, the presence of cytologic atypia in association with atypical junctional nests (showing short interconnections or characterized by nonhomogeneous cellularity) was suggestive of dysplasia, while widespreading pagetoid cells scattered in the epidermis or atypical cells diffused throughout the DEJ associated with nonedged papillae were tipically found in melanoma (Pellacani et al. 2012, e109-21)

A focus on pink and nodular lesions

Many studies regarding pigmented lesions, showing bright components due to the high content of melanin, have been performed, while hypopigmented/amelanotic lesions were not so extensively investigated. A stepwise approach was described in order to study hypopigmented/amelanotic lesions (Longo et al. 2015, 31–41). Initially, the distinction between inflammatory versus skin cancer has to be formulated. Then, RCM evaluation can be helpful in determining the nature of the lesion, underlining the importance of identifying specific morphological clues in order to classify nonpigmented skin neoplasms into melanocytic versus nonmelanocytic and benign versus malignant. More specifically, the presence of a nested melanocytic proliferation at the DEJ or dermis level allowed to ascribe a given lesion as melanocytic; the presence of basaloid bright tumour islands was a key RCM feature for the diagnosis of BCC; and an epidermal disarrangement associated with small demarcated papillae was suggestive for the diagnosis of SCC (Longo et al. 2015, 31–41).

The second issue, depth exploration, was analyzed in a retrospective study involving 140 nodular lesions. Nodular neoplasias belonging to both melanocytic (among which 23 "pure" nodular melanomas [NMs], nine melanoma metastasis) and nonmelanocytic tumours (among which 28 BCCs and six invasive SCCs) have been explored, concluding that RCM was a valuable tool for nodules because, in many cases, a thinned epidermis allowed a good visualization of peculiar diagnostic structures located in the superficial dermis (Longo et al. 2013, 58–67). The key concept of this study was that cases characterized by the absence of criteria should lead to prompt excision because a deep malignant tumour proliferation cannot be assessed and thus melanoma diagnosis cannot be ruled out.

RCM updates for examination of melanoma

Melanoma is one of the tumors with the highest rising in incidence. Only an early detection of melanoma can led to a better prognosis (Garbe and Leiter 2009, 3–9).

The original histopathologic classification of melanoma is based on growth pattern and biologic behavior and it included LM/LMM, superficial spreading (SSM) and NM. Then, other types of melanoma have been added to this classification (Clark et al. 1969, 705–27).

Superficial Spreading Melanoma (SSM)

SSM is the most common type of melanoma in Caucasian (Jemal et al. 2008, 71–96). It is characterized by a radial growth in the initial phase, followed by vertical proliferation after invasion. A 3-steps model of progression in SSM has been described (Scope et al. 2008, 1644–49). The first step was the proliferation of single cells or melanocyte nests along the DEJ occurring in the rete ridges, in the suprapapillary plate and in suprabasal layer of epidermis, then a remodeling with a progressive flattening of DEJ associated with inflammation, angiogenesis and fibroplasia followed by dermal invasion. This means that to each step corresponded an increased degree of atypia. Pagetoid cells, mild to moderate cytological atypia and non-edged papillae can be described in superficial lesions (Gareau et al. 2010, 61713). With increasing thickness, epidermal disarrangement, cell pleomorphism, progressive papillary infiltration with bright cells or cerebriform melanocyte nests can be added to the list of features observed (Pellacani, Cesinaro and Seidenari 2005, 469–74).

In particular, considering epidemiologic and RCM characteristics, SSM included: pagetoid and solar melanoma (Longo, Casari and Pellacani 2012, 151–78). Pagetoid melanoma was described in adults with a history of intermittent solar exposure and several nevi. Upon dermoscopy, reticular, globular, multicomponent or nonspecific pattern could be identified. Histologically, this tumor is typified by an intraepidermal growth, corresponding to RCM findings of pagetoid infiltration (Pellacani, Cesinaro and Seidenari 2005, 532–37) of the epi-

dermis that showed an atypical honeycomb or cobblestone or fully disarrayed pattern. Pagetoid cells were mainly roundish and distributed throughout the lesion or located at the periphery (Figure 1A, B). When centrally located, a differential diagnosis with Spitz, congenital, traumatized and dysplastic nevi should always be considered (Pellacani et al. 2009, 236–47). DEJ was predominantly represented by meshwork with edged and nonedged papillae with admixed atypical cells. Going deeper to the upper dermis, melanocytic homogeneous nests or, in over the half of cases, dense and sparse nest were prevalent. Inflammation presented as plump bright cells and bright particles (Longo, Casari and Pellacani 2012, 151–78).

Melanoma on sun-damaged skin is located by definition in the most sun-exposed areas of patients with a low nevus count. Dermoscopically regression features such as white combined with blue-gray color and/or peppering. Histologically, atypical melanocytes in the basal layer and a lymphocytic infiltrate were commonly presented in the superficial portions of the dermis while the surrounding skin showed photodamage. Peculiar RCM findings were pagetoid cells with variable shape, usually dendritic, mainly located around the hair follicle, in the context of an atypical or disarranged epidermis. At the DEJ, ringed or meshwork or uneven pattern could be observed in association with atypical cells. Upper dermis was predominantly characterized by solar elastosis and inflammation. Polycyclic papillary contours and bulbous projections, sometimes detectable at the periphery of the lesion could led to a misdiagnosis with a benign lesion, underlying the importance of a fully evaluation of the lesion (Longo, Casari and Pellacani 2012, 151–78).

Nodular Melanoma (NM)

NM is the most aggressive type of melanoma (Chang, Karnell and Menck 1998, 1664–78). A difference should be made between vertical growth phase in a context of radial growth phase of SSM and "pure" NMs, these latter representing a separated subgroup of melanomas (Segura et al. 2008, 1311–20).

Pigmented NMs usually appeared as symmetric lesions, with a homogeneous blue pigmentation, showing also a blue-white veil, pink and/or black color and milky-red/pink areas with large-diameter vessels predominantly located in the periphery, with pure NMs showing less dermoscopic features than SSMs with a vertical growth phase (Menzies et al. 2013, 699–709).

Table 1. RCM criteria for the evaluation of melanocytic lesions

RCM criteria	
Epidermis	
Regular honeycombed pattern	Large polygonal cells with dark nuclei and bright cytoplasm and cell borders
Regular cobblestone pattern	Small polygonal cells with refractive cytoplasm separated by a less refractive border
Atypical honeycombed pattern	Irregularity in size of the cells and thickness of the contour within a honeycombed structure
Atypical cobblestone pattern Broadned epidermal pattern	Irregularity in size and/or refractivity of the cells within a cobblestone structure Polygonal cells with demarcated, bright borders and black nuclei
Epidermal disruption	Disarray of the normal architecture of superficial layers with unevenly distributed bright granular particles and cells, in absense of honeycombed or cobblestone pattern
Acanthosis	Prominent bright epidermis intermingled with papillae
Ulceration	Dark areas, usually with sharp borders and irregular contours, filled with amorphous material and/or clotted bright small particles
Pagetoid cells	Large roundish nucleated cells, twice the size of basal keratinocytes, with a dark nucleus and bright cytoplasm, with different number and distribution
Dendritic pagetoid cells	Large cells with bright cytoplasm and dark nucleus with clearly visible dendrites connected to the cell
Roundish pagetoid cells	Large bright cells with well outlined border and dark nucleus within the epidermis, represent the most common finding for melanoma diagnosis
Hyporeflective pagetoid cells (HPCs)	Large cells, round to oval in shape, appearing as well-demarcated structures within the epidermal layer
Striking pleomorphism	Variability of the aspect of pagetoid cell and/or the presence of cells with bizarre shapes
Dermoepidermal junction (DEJ)	
Ringed pattern	Densely packed bright rings corresponding to papillae surrounded by a rim of small bright cells sharply contrasting with the dark background
Junctional nests	Compact, round to oval bright cell aggregates, connected with the basal layer of the epidermis
Sheet-like strucure	Cells distributed at the transition of the DEJ showing loss of dermal papillae not aggregated in clusters but closely distributed in the same plane with the loss of dermal papillae
Edged papillae	Dermal papillae demarcated by a rim of bright cells, appearing as bright rings sharply contrasting with the dark background
Non-edged papillae	Dermal papillae without a demarcating bright rim at the DEJ
Atypical cells	Large cells showing a bright cytoplasm with clearly outlined borders and sharply contrasted dark nucleus inside, roundish to oval in shapes, sometimes presenting dendritic-like structures, showing different number and distribution
'Medusahead-like' structure.	Dendritic and pleomorphic cells aggregated into clusters bulging from the hair follicle
Upper dermis	
Dermal dense nests	Compact aggregates with sharp margin in which outline of individual cells is indiscernible or similar in shape, size and refractivity
Dermal dense nonhomogenous nests	Compact cell aggregates showing non homogeneity in cell morphology and reflectivity
Dense and sparse dermal nests	Cell aggregates with irregular, discohesive margins showing isolated nucleated cells at the periphery
Cerebriform clusters	Confluent amorphous brain-like aggregates of low reflecting cells exhibiting granular cytoplasm without evident nuclei and ill-defined borders, showing a fine hyporeflective "fissure" like appearance
Striking pleomorphism within nests	Nonreflecting structures with a well-demarcated border, containing isolated round to oval cells with dark nucleus and reflecting cytoplasm, with bizarre shapes
Inflammation	Large irregularly shaped bright cells with ill-defined borders and usually no visible nucleus which can be visible within dermal papillae
Collagen	Bright elongated fibrillar structures with no cellular component, no visible nucleus and movement, distributed within the dermis, arranged in a reticulated network or as bright bundles

Histologically, a flattening of the epidermis and eventual ulceration overcoming the nodular component could be found whereas a cohesive nodule or smaller nests of malignant melanocytes with a pushing or expansive pattern of growth at the dermis level were found. Upon RCM, "epidermal consumption" was detected while acquiring RCM image stacks in NMs. This feature was characterized by a rapid of transition from epidermal to dermal compartment, due to the thinning of the epidermis down to two to three layers of keratinocytes (Hantschke, Bastian and LeBoit 2004, 1621–25).

Epidermal layers were characterized by regular honeycombed or, sometimes, polygonal cells with demarcated, bright borders and black nuclei forming a broadened honeycombed pattern. Pagetoid cells, with either a roundish or dendritic shape, were identified in vertical growth phases whereas in NMs the pagetoid

infiltration was not detectable or focally represented.

Ulceration represented a common finding, appearing as dark areas, usually with sharp borders and irregular contours, filled with amorphous material and/or clotted bright small particles. Ulcerations covering over than one half of the surface of the lesion hampered penetration of RCM light and visualization of underlying structures. At the DEJ, typical dermal papillae were absent and the basal layer was composed of pleomorphic cells, arranged in sheet-like structures.

Within the dermis, cerebriform melanocyte nests with prominent cellularity and moderate inflammatory infiltrate with the absence of regression were identified. Cerebriform nests, showing up as hyporefrective aggregates of small cells outlined by bright collagen septae, were considered highly specific of malignancy and suggestive of invasion of the deepest part of dermis in NMs (Figure 1C, D) (Segura et al. 2008, 1311–20). Interestingly, RCM features found within dermal component of nodules arising from a SSM or to pure NMs were not distinguishable.

Furthermore, vascularization was found to be an important clue at this stage of invasion. Convolute and tortuous vessels, in proximity of tumoral proliferation or even crossing malignant cell growth, were frequently decribed. Live imaging unrevealed the presence of vessels with large caliber and fast blood flow.

Recently, Longo et al. (Longo et al. 2015, 31–41) described the role of RCM in the evaluation of 140 nodular lesions including NMs, melanoma metastasis and other benign and malignant skin lesions.

Considering that the nodular portion of NMs shared morphologic findings with metastasis, these two entities where grouped in the discriminant analysis, highlighting that widespread pagetoid distribution, many atypical cells and cerebriform nests were peculiar elements for the diagnosis of melanocytic malignancy.

Whitmore, confocal microscopy reached 96.5% sensitivity and 94.1% specificity in the diagnosis of NMs and melanoma metastasis versus all other nodular lesions, regardless of the limited laser depth penetration of RCM. Limitations were represented above all by wide ulcerations or marked hyperkeratosis (Longo et al. 2015, 31–41). This means that, once again, biopsy and histopathological examination should always be performed in doubtful cases.

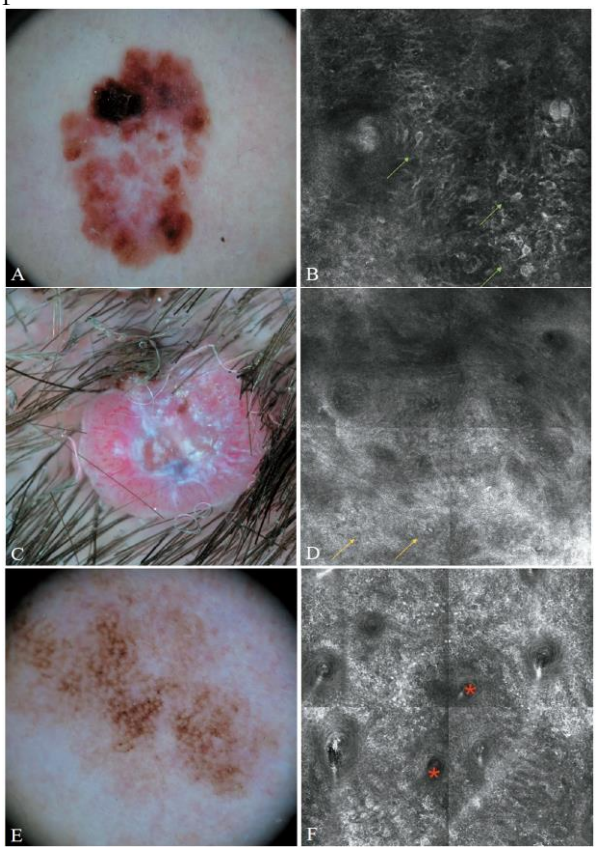

Figure 1. Dermoscopic and confocal pictures of melanomas. A) Dermoscopic image of superficial spreading melanoma. It presents multi component pattern with central area of regression, eccentric hyperpigmentation, inverse network; B) confocal image of melanoma. Several roundish pagetoid cells (green arrows) within the epidermis with a consumption of he epidermis itself; C) dermoscopic image of nodular amelanotic melanoma showing irregular vessel, central ulceration; D) confocal image of nodular melanoma showing the presence of cerebriform nest (yellow arrows) in the dermis. These nests are rarely notices in melanoma because they are too deep in the dermis but they are typical of more invasive melanoma; E) dermoscopic image of lentigo maligna (LM) of left cheek. A hyperpigmentation around follicular openings can be noticed; F) confocal image of LM with numerous dendritic pagetoid cells around follicular openings (red asterisks). This is a typical sign of LM (Guida et al. 2015, 547–63).

Lentigo Maligna (LM) / Lentigo Maligna Melanoma (LMM)

LM and LMM are melanomas mainly occurring on the face and other sun-exposed areas. Dermoscopy identified several peculiar aspects like asymmetrical follicle pigmentation, annular granular pattern, pigmented rhomboidal structures and/or obliterated hair follicles (Pralong et al. 2012, 280–87). RCM examination of LM/LMM revealed a significant similarity to solar SSM. Epidermal disarrangement with the presence of numerous round or dendritic ple-

omorphic cells, distributed in all of its layers, was reported (Langley et al. 2006, 88–97). An important clue was the presence of atypical cells grouping around the hair follicle (Langley et al. 2006, 88–97; Tannous et al. 2002, 260–63). In the papillary dermis, the presence of melanophages and reticulated bright collagen bundles, ascribable to solar damage were observed.
Nests of atypical and nucleated melanocytes could also be seen and these structures corresponded, coming back to dermoscopy, to globules (de Carvalho et al. 2016, 878–80).
In a recent study (de Carvalho et al. 2015, 128–33), RCM examination of 60 lesion revealed that the most relevant differences between the nonmelanoma skin neoplasms and melanomas involved the presence of atypical cells both at th junction and spreading upwards in a pagetoid fashion, usually with a dendritic morphology (Figure 1E, F). Furthermore, a meshwork pattern was observed in LM/LMM, compared with the most common ringed pattern in association with polycyclic contours in benign lesions. An important clue, known as 'Medusahead-like' structures, characterized by elongated buddings bulging from the hair follicle and populated by dendritic/pleomorphic cells, was found in 11 of 30 LMs of the case series analyzed, usually in relation to asymmetric follicular pigmentation and/or around hyperpigmented follicles, whereas folliculotropism of atypical cells was detected in one half of cases.
Another important aspect concerned the helpfulness of RCM in the analysis of hyperpigmented areas, suspicious for malignancy (Tran, Wright and Cockerell 2008, 852–71). In fact, when evaluating each dermoscopic pattern in lesion with different biological nature, RCM substrates helped in providing the correct diagnosis avoiding a skin biopsy (de Carvalho et al. 2015, 128–33).
Noteworthy, suggestive RCM features for LM can be found in early melanomas, providing differential diagnosis from solar lentigo, seborrhoeic keratoses and lichen planus-like keratosis (de Carvalho et al. 2015, 128–33). Finally, different attempts have been made in order to define margins of LM/LMM more accurately using RCM (Champin et al. 2014, 247–56; Guitera et al. 2013, 692–98). Preoperative mapping is extremely important because these lesions often show ill-defined margins, not easily detectable with the naked eye and with the help of dermoscopy. The assumption is that a careful preoperative mapping guiding surgical excision could led to a reduction of local recurrences. Intuitively, the analysis of LMs/LMMs with the handheld RCM device Vivascope 3000 can be useful in difficult-toexplore sites and in lesions bigger than 8x8 mm.

Amelanotic melanoma

The diagnosis of amelanotic melanoma (AM) has been challenging clinically and instrumentally (Guitera et al. 2013, 692–98). RCM features described for SSM and NM can also be applied for AMs (Longo et al. 2015, 31–41). Nevertheless, considering that melanin and melanosomes usually create a source of contrast, (Stoecker and Stolz 2008, 1207–10) the identification of clues became fundamental. Interestingly, Losi et al. (Losi et al. 2014, 48–54) detected a new confocal feature called hyporeflective pagetoid cells (HPCs). HPCs were described as large cells, round to oval in shape, appearing as well-demarcated structures within the epidermal layer. At a higher magnification, these structures, with a particular shape and size, presented a dark round central area corresponding to the nucleus, within a dark grey grainy material corresponding to the cytoplasm.
A difference should be operated between flat and papular/nodular lesions. Flat AMs were characterized by honeycombed pattern in association with HPCs, single or organized in nests, and dendritic-shaped particles corresponding to melanocyte or Langerhans cells (Hashemi et al. 2012, 452–62). At the DEJ level, different architectural patterns were identified: nonedged dermal papillae, nonspecific pattern with a loss of dermal papillae, due to the flattening of the DEJ or a meshwork pattern with focal or wide spreading irregular junctional nests composed by atypical melanocytes (Pellacani et al. 2014, 414–18). In upper dermis melanocyte nests were usually less bight than nests belonging to pigmented tumors.
In palpable-to-nodular AMs, honeycombed epidermal pattern with a more evident brightness of the intercellular borders of keratinocytes was observed.

HPCs were found more commonly in SSM with a nodular component than in pure NMs, like pagetoid cells in pigmented NMs (Longo et al. 2013, 58–67).

As previously described for pigmented NMs, ulceration was a common finding also in nodular AMs while cerebriform nests, although rare and presenting a weak reflectivity, represented the most important clue for the diagnosis of NM (Segura et al. 2008, 1311–20).

In order to validate this feature, a retrospective study has been performed, including 20 amelanotic MMs and controls represented by 10 melanocytic nevi, 20 hypo/nonpigmented nonmelanocytic

lesions and 20 pigmented melanomas. HPCs were abundantly found in 85% of AMs. They were also described in Spitz nevi. In these cases they were correlated with pagetoid infiltration of hypomelanotic melanocytes on histopathology. This is an important finding when considering that few HPCs were also highlighted in nonmelanocytic lesions (three SCCs, two seborrhoeic keratoses and one BCC), corresponding to enlarged or dyskeratotic keratinocytes by histopathology (Losi et al. 2014, 48–54).

Mucosal melanoma

This is a rare form of melanoma. Although most mucosal pigmented macules are benign, it can be clinically and dermoscopically challenging to rule out an early melanoma, above all when considering that most cases of malignancy in oral mucosa and anogenital are relatively advanced at diagnosis.

Early detection and appropriate treatment significantly increases survival. RCM findings for this type of melanoma included: roundish cells, a high density of atypical dendritic cells and intraepithelial bright cells in a case series including labial or genital pigmented macules among which 10 macular melanomas (Debarbieux et al. 2014, 1276–84).

Toward a confocal-based classification of melanoma

A previous report 4 described a RCM-based diagnostic algorithm consisting of 2 major and 4 minor criteria to evaluate the degree of atypia of melanocytic lesions. Major criteria (scoring two points each) included non-edged papillae and the presence of atypical melanocytes. Minor criteria (scoring one point each) included pagetoid cells in the epidermis, wide spreading pagetoid cells, cerebriform melanocytic clusters, and individual, nucleated melanocytes within dermal papillae. A score of ≥ three points led to a confocal-based diagnosis of melanoma.

A web-based multicenter study involving RCM users from 6 different countries, evaluated characteristic features of 55 melanocytic nevi, 20 melanomas and other nonmelanocytic skin neoplasms. The discriminant analysis of these lesions identified three important features for melanoma diagnosis. Pagetoid cells spreading within epidermis, atypical cells at the DEJ and irregular architecture of epidermis; these latter showed a low interobserver reproducibility (Farnetani et al. 2015, 1075–80).

Recently, Pellacani et al. (Hashemi et al. 2012, 452–62) have described a confocal- based classification. The study included 100 melanomas, of which 11 LMs, nine LMMs, 74 SSMs (15 of which in situ) and six NMs. Dendritic and round cell, dermal nest, combined and non-classifiable melanomas have been described, depending on prevalent RCM features.

Dendritic cells were found in large melanomas showing a pigmented network in subjects with a low number of atypical nevi. Dendritic cells characterized a slow-growing group of melanomas, described by Argenziano et al. (Argenziano et al. 2010, 267–73) in which round cells and dermal nests could also be found with the progression of the disease. This observation allowed to formulate the hypothesis that the initial growth of melanoma could be represented by intraepidermal proliferation of dendritic melanocytes that, when de-differentiating, could leave the place to cells with a roundish shape and invasive capability (Zalaudek et al. 2008, 1375–79). LMs and SSMs were predominantly observed in this group of melanomas (Pellacani et al. 2014, 414–18).

Round cells were commonly associated with thicker but smaller melanomas compared to dendritic ones, occurring in patients with many atypical nevi. The degree of invasiveness was variable. The next step for melanoma progression could be exemplified by a further cell de-differentiation in order to create less cohesive cells aggregated into dense-andsparse clusters and/or cerebriform nests (Pellacani, Cesinaro and Seidenari 2005, 469–74).

Dermal nests typified NM, characterized by a rapid pattern of growth, a deep dermal invasion, limited epidermotropism and poorly differentiated melanocytes, (Zalaudek et al. 2008, 1375–79) while combined melanomas were the biggest ones. Nonclassifiable type melanomas showed ringed architecture with few atypical cells, usually roundish; these were supposed to represent an early phase of round cell melanomas (Pellacani et al. 2014, 414–18).
Finally, Longo et al. (Longo et al. 2013, 941–45), used RCM in support to dermoscopy and histopathology, and described a new melanoma entity called "nested melanoma of the elderly", characterized by the presence of a clod pattern in which compact nests with variable atypia.

RCM for the examination of NMSC

Skin cancer is the most commonly diagnosed cancer in the Caucasian population, with rapid further increasing incidence rates (Rubin, Chen and Ratner 2005, 2262–69). SCC and BCC are considered as NMSCs. The incidence ratio between those two NMSC types is approximately 1:4, while in immunosuppressed patients this ratio is inverted (Ridky 2007, 484–501; Lomas, Leonardi-Bee and Bath-Hextall 2012, 1069–80). RCM criteria for NMSCs are summarized in Table II.

Table 2. RCM criteria for the diagnosis of non-melanocytic skin cancer
(NMSCs)

RCM criteria	
Basal cell carcinoma	
Tumor islands	Round to oval, cord-like or lobulated structures at the level of DEJ or superficial dermis that can be either darker than the surrounding epidermis or dermis ("dark well-demarcated structures silhouettes") or bright well-demarcated structures
Polarization of nuclei (streaming)	Cells within the tumor islands, or overlying basal or spinous keratinocytes, display nuclei that are elongated and distorted into alignment along the same axis
Dark cleft (clefting)	Dark slit-like space observed between tumor island and surrounding dermis
Dendritic cells	Bright delicate, dendritic structures within bright tumor islands or in the overlying epidermis
Plump-bright cells	Oval to stellate cells with indistinct borders and without apparent nucleus in the dermis
Canalicular blood vessels	Thickened, elongated or tortuous dark structures, oriented parallel to the skin surface, containing moving small, round bright structures (white blood cells)
Fenestrated pattern	Tumor islands or cords with palisading cells at the periphery, which outline hyporefractile 'holes' corresponding to the fibrous stroma
Actinic keratosis and squamous cell carcinoma	
Parakeratosis	Individual highly-refractile round cells in the stratum corneum
Scale (hyperkeratosis)	Increased thickness of stratum corneum seen as refractile amorphous material
Irregular (atypical) honeycomb pattern	Abnormal pattern of the spinous-granular layers formed by bright cellular outlines
Solar elastosis	Moderately refractive lace-like material adjacent to collagen bundles
Inflammatory infiltrate	Small highly refractile cells within the epidermal layers and the superficial dermis
Round blood vessels	Dilated blood vessels within the dermal papillae that run perpendicular to the horizontal RCM plane of imaging.

Confocal patterns and characteristics of BCC

BCC is the most common skin cancer worldwide, mainly affecting fair-skinned adults. BCC is a slow-growing tumor that rarely metastasizes. It is one of the most studied skin cancers upon RCM, and this can be related to its high incidence. Clinically, BCC is characterized by pink to red-brown patches or papules, central erosion or ulceration with or without the presence of crusts, a pearly shine and a raised border with the presence of telangiectasias (Webber et al. 2011, 179–85).
Some differences in diagnostic accuracy have been evaluated in a recent study involving 54 lesions of which 45 histologically proven BCCs. Castro et al. (Castro et al. 2015, 1164–69) highlighted a high positive predictive value obtained with both RCM probes, while a higher negative predictive value was associated with the use of the traditional wide probe, probably because its broader field-of-view allows a more exhaustive search for BCC criteria.
Several dermoscopic patterns were identified but they will be taken into account for BCC subtype classification that follows. On histopathology, most BCC subtypes show aggregates of atypical basaloid cells with peripheral palisading of nuclei, stroma with fibroplasia, and frequently clefting between tumor aggregates and stroma.
González et al. firstly described five relevant criteria for the confocal diagnosis of BCC, which were later validated in a larger study (González and Tannous 2002, 869–74; Nori et al. 2004, 923–30). These criteria included: elongated monomorphic nuclei, polarization of these nuclei along the same axis, a prominent inflammatory infiltrate, increased dermal vasculature and pleomorphism of the overlying epidermis.
Later on, further descriptors were added, such as the presence of tumor islands and cords considered the RCM hallmark of the tumor. These structures were characterized by a bright outline and clearly defined shape, especially in the presence of pigmentation.
However, they were usually clearly visible also in hypopigmented tumors, although less contrasted.

Dark silhouettes represented a different morphologic presentation of BCC tumor islands, corresponding to hypopigmented tumor proliferation visible as a dark, footprint-like shadow in a context of bright compact collagen (Figure 2A, B) (Agero et al. 2006, 638–43; Segura et al. 2007, 883–86; Scope, Mecca and Marghoob 2009, 106–7; Braga et al. 2009, 230–41; Segura et al. 2009, 216–29; Ulrich et al. 2011, 190–95; Casari et al. 2011, 406859).

Additionally, the presence of dendritic cells in BCC nests was correlated with melanocytes, typically found in pigmented BCCs where they appeared as long dendritic shaped cells entrapped inside the tumor islands (Segura et al. 2007, 883–86). Along with melanocytes, the pigmentation upon RCM was related to the presence of inflammatory infiltrate showing up as bright spots or to ill-defined plump bright cells corresponding to melanin-rich melanophages (Figure 2C, D). (Guitera et al. 2012, 2386–94).

Blood flow was typically altered in BCC and was described as an elongation of blood capillaries, which were increased in number and size.

In a recently published large prospective study, Guitera et al. (Guitera et al. 2012, 2386–94) introduced a new concept called "epidermal shadowing", described as a large dark featureless area disrupting the epidermis due to en face clefting of the underlying tumor nests.

RCM subtypes of BCC

The identification of different subtypes of BCC is important in order to choice the most appropriate treatment and to determine surgical excision margin.

BCC can be divided into 3 main subtypes based on the histopathologic growth pattern: nodular (nBCC), infiltrating (iBCC) and superficial (sBCC). Longo et al. (Longo et al. 2014, 716–724.e1) presented a study to identify specific criteria to differentiate subtypes of BCC thanks to the retrospective analysis of 88 lesions, including 44 sBCCs, 22 nBCCs, and 22 iBCCs.

Firstly, different BCC subtypes were differentiated dermoscopically being sBCC characterized by fine telangiectasias, multiple small erosions, and structures corresponding to dermoepidermal pigmentation. In contrast, the detection of ovoid nests, arborizing vessels and large ulcerations were associated with nBCCs or iBCCs. iBCC has been reported to have a peculiar dermatoscopic pattern, consisting of shiny white-red structureless areas and arborizing vessels of a smaller caliber and less tendency to branch into finer capillaries compared to those seen in nBCC tumors (Lallas et al. 2014, 303–11). Upon RCM, the presence of cords connected to the epidermis was significantly associated with sBCC. nBCC was primarily typified by the presence of large tumors nests.

Furthermore the presence of clefting was much more frequent in nBCC compared to sBCC and iBCCs.

Finally, although increased vascularization was detected in all subtypes, the caliber of vessel was larger in nBCC compared to sBCC and iBCC. iBCC was characterized, upon RCM, by the presence of dark silhouettes and abundant bright compact collagen.

Moreover, iBCC was the most common diagnosis in presence of dark silhouettes and in absence of small tumor islands, big tumor islands, and cords connected to the epidermis. Peripheral palisading was present in the majority of tumors of all subtypes, while inflammation was less frequently observed (Horn et al. 2008, 620–25).

A previous study (Peppelman et al. 2013, 255–62) involved also micronodular (mnBCC) and mixed-type variants. 27 patients with 43 biopsied lesions with a histopathological diagnosis of BCC were included. Of these, 23 were sBCC, 11 nBCC, three mnBCC and six mixedtype BCC.

Tumor nests with peripheral palisading, branch-like structures, fibrotic septa and increase in vascular caliber were the main RCM features for nBCC and mnBCC. Then size, shape and location of the tumor nests allowed a differentiation between nBCC and mnBCC. Solar elastosis and the location of the tumor nests just below or in connection with the basal cell layer characterize sBCC. Criteria for iBCC were not easily detectable in this study because mixedtype but not "pure" iBCC were included (Peppelman et al. 2013, 255–62).

Another variant that should be mentioned is fibroepithelioma of Pinkus (FeP), representing an unusual form of basal cell carcinoma, which may clinically mimic a range of benign skin tumors that are not routinely excised. 20 published cases of FeP reported in the literature

were reviewed, suggesting that dermoscopy and RCM could help the clinical diagnosis and, consequently, management of FeP (Reggiani et al. 2013, 207–11). On characterhistopathology, FeP showed tumor islands and anastomosing strands of basaloid, often palisading cells, included in a fibromatous stroma. Upon RCM, the analysis of 9 cases (6 nonpigmented and 3 pigmented) revealed the presence of a "fenestrated pattern", consisting of tumor islands or cords with palisading cells at the periphery, which outline hyporefractile 'holes' corresponding to the fibrous stroma (Longo et al. 2012, 556).

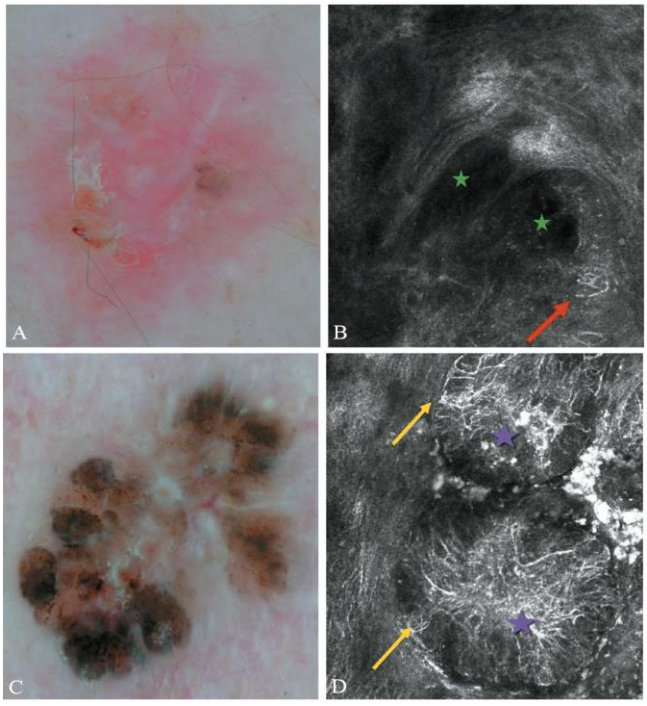

Figure 2. Dermoscopic and confocal pictures of basal cell carcinomas (BBCs). A) dermoscopic image of invasive BCC, lightly pigmented. There are multiple ulcerations, fine multiple telangiectasia and blue-grey dots; B) confocal image (square 0.5x0.25) showing typical features enabling the diagnosis: dark silhouette (green star) and bright filaments corresponding to melanophages (red arrow) that create the light pigmentation visible dermoscopically; C) dermoscopic image of nodular pigmented BCC: grey-blue ovoidal nest, leaf-like maple; D) confocal image of multiple tightly packed (violet stars) and numerous melanophages (yellow arrows). The major number of melanophages is related to the pigmentation more evident in this BBC (Guida et al. 2015, 547–63).

Confocal patterns and characteristics of AK

AKs represent the earliest stage in the development of SCC and relevant biomarkers for individuals at risk for development of invasive SCC.

The main risk factors are fair skin, intermittent or prolonged exposure to UV radiation, genetic predisposition, and immunosuppression (Ulrich et al. 2008, 610–19). Usually the diagnosis of AK is based on clinical examination, but biopsies are routinely performed in order to rule out invasive SCC or other skin diseases like porokeratoses, seborrheic keratoses, basal cell carcinoma or Bowen's disease. Dermoscopy has recently been applied for the diagnosis of AK, the classic features include the presence pink-to-red "pseudonetwork" surrounding the hair follicles, scale, fine and linear
vessels surrounding the hair follicles and keratotic plugs within the hair follicles (Horn et al. 2008, 620–25). Histopathological features included: proliferation of atypical keratinocytes AKs, starting at the basal cell layer, inducing an architectural disarray due to basal crowding of keratinocytes with different degrees of involvement of epidermis. Mitoses and nuclear pleomorphism represented a common finding together with parakeratosis alternated with orthokeratosis at the follicle ostia in the stratum corneum, solar elastosis and inflammation in the dermis.
Confocal laser microscopy could be useful in order to perform the correct diagnosis. First of all, keratinocytes in the stratum corneum appeared detached from one another and are seen as highly refractive polygonal structures. Moreover, nuclear retention, corresponding to parakeratosis in histology, was visualized as dark round structures in the center of the corneocytes. RCM images showed an atypical honeycomb pattern (disruption of the characteristic honeycomb pattern) and cells with dark nuclei of irregular shapes and sizes (Figure 3A, B). Images of the superficial dermis showed different degrees of solar elastosis, which is visualized as bundles of lace-like collagen (Horn et al. 2008, 620–25).

Confocal grading of AKs

Several options are nowadays available for the treatment of AKs. This means that specific patterns corresponding to different grades and subtypes may aid to choose the most appropriate treatment and in monitoring of the response to the therapy. Interestingly, preliminary confocal microscopy data based on keratinocyte atyp-

ia allowed the creation of a grading of AK. Grade 1 AK presented focal areas of atypical honeycombed pattern at the level of the stratum spinosum, intermingled with areas of preserved, typical honeycombed pattern. In grade 2 AK, the atypia of keratinocytes was more diffuse, involving the stratum spinosum and granulosum. Keratinocytes presented a marked atypia, with different sizes and shapes of the cells. Grade 3 AK was characterized by a markedly atypical honeycombed pattern with areas of partial disruption of the normal epidermal layers, defined as a disarranged pattern. Pleomorphic keratinocytes showed a wide variability in cellular size and shapes, and irregular intercellular keratinocyte connections were detected (Zalaudek et al. 2014, 80–87).

Confocal patterns and characteristics of SCC

SCC is a common skin tumor derived from epidermal keratinocytes. It typically presents as a red scaling plaque, with or without ulceration. SCC is complicated by its metastatic potential (Soter, Wilkinson and Fitzpatrick 1973, 296–302). Dermoscopy features of SCC were dotted or glomerular vessels, when present, and a scaly surface that, when too wide, could impair the visualization of vessels (Zalaudek et al. 2004, 1112–16).

Histopathologically, crowded, enlarged, and pleomorphic nuclei, dykeratosis, and parakeratosis were typically found in SCCs (Ackerman and Mones 2006, 9–22). Bowen disease (BD) is SCC in situ that is histologically characterized by a proliferation of atypical pleomorphic keratinocytes throughout the epidermis.

RCM features of keratinocytic atypia have been described in recent studies (Horn et al. 2008, 620–25; Ulrich et al. 2008, 610–19; Rishpon et al. 2009, 766–72), but nowadays no detailed description of RCM features of BD exists in the literature.

Ulrich et al. (Ulrich et al. 2012, 451–53) recently published a study of 10 BD. The most common confocal findings were disruption of the stratum corneum, an atypical honeycomb pattern in the epidermis with a greater degree of architectural disorder and cellular atypia than in AK (Figure 3C, D), S-shaped blood vessels in the center of the dermal papillae, and two types of characteristic targetoid cells. The first type were large cells with a dark center, a bright rim, and a dark halo, and the second type were large cells with a bright centerand a dark halo. Cells are thought to correspond to the different degrees of dyskeratosis seen by conventional histology. Other features observed were parakeratosis, multinucleated cells, and solar elastosis.

In 2009 Rishpon et al. (Rishpon et al. 2009, 766–72) published a study of the confocal characteristics of 38 clinically suspected SCC lesions. The features identified were an atypical honeycomb or disarranged pattern in the epidermis (Figure 3E, F), rare round cells with nuclear atypia in the stratum spinosum and stratum granulosum, and round blood vessels crossing the dermal papillae.

RCM images of the stratum corneum typically revealed bright amorphous structures corresponding to the presence of crusts on the tumor surface and polygonal nucleated cells with a bright rim around a dark nucleus (parakeratosis). Hyperkeratosis and ulceration could impair the evaluation of the lesion.

Currently, the diagnostic distinction between AK and SCC, especially when solely based on clinical aspects, may not always be easily. Obtaining biopsies is an invasive method and it is not always possible to do. Peppelman et al. (Peppelman et al. 2015, 1302–9) try to assess in vivo RCM features that are specific enough to make a distinction between AK and SCC using RCM as a non-invasive in vivo diagnostic method. They demonstrated that the presence of architectural disarray in the stratum granulosum in combination with architectural disarray in the spinous layer and/or tumor nest in the dermis were the main RCM features to distinguish SCC from AK.

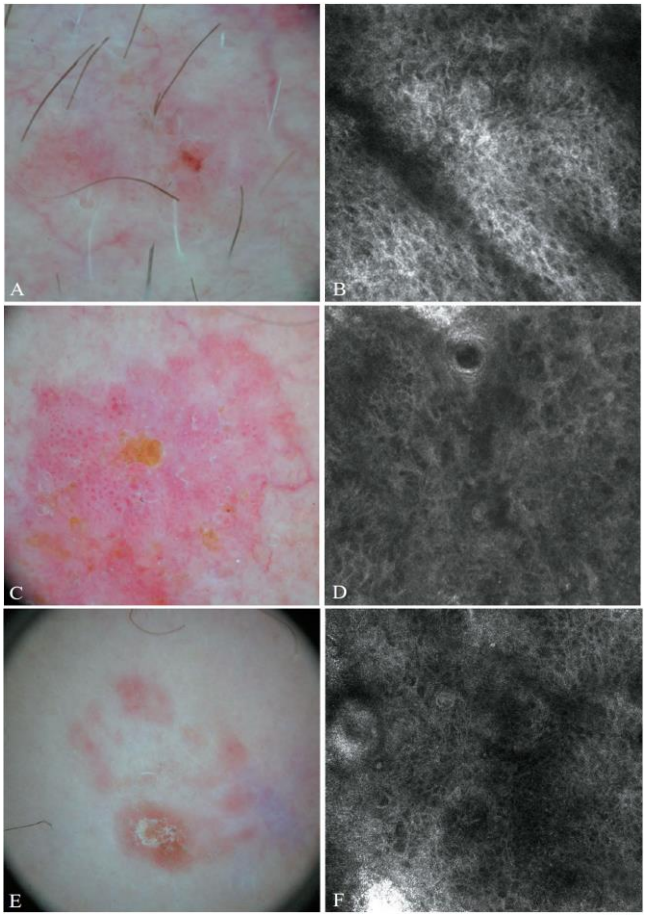

Figure 3. Dermoscopic and confocal pictures of aktinic keratosis (AKs) and squamous cell carcinoma (SCCs). A) Dermoscopic image of AK with strawberry pattern; B) confocal image of the epidermis showing a light atypical honeycombed, with the presence of irregular keratinocytes for shape and size; C) dermoscopic image of Bowel disease showing scales and glomerular vessels; D) confocal image showing mild irregular honeycombed; E) dermoscopic image of invasive squamous cell carcinoma showing scales, erosion and atypical vessels; F) confocal image showing a severe atypical honeycombed typical of SCC (Guida et al. 2015, 547–63).

From in vivo to ex vivo

Advantages related to in vivo RCM consist of real-time observation, underlying different clinical applications of RCM in the evaluation of melanocytic and non-melanocytic lesions, and in particular in the study of facial maculae and amelanotic/hypopigmented tumors and management of subclinical margins, recurrences, or monitoring noninvasive treatment of tumors (Castro et al. 2015, 1164–69; Fraga-Braghiroli et al. 2014, 933–42; Cinotti et al. 2014, 912–918.e2; García-Hernández et al. 2013, 205256).

In the last few years a new technique became available: ex vivo fluorescence confocal microscopy (FCM). FCM enables real-time imaging of skin morphology directly in freshly excised tissue. For this reason, the application of interest is rapid detection of residual BCC in skin excisions during Mohs surgery (Bennàssar et al. 2014, 360–65) .

FCM requires a contrast agent that is able to highlight nucleated cells; this means that with the use of this technique only a few fluorescence is collected from the dermis while one of limit of RCM is the reflectance coming from the dermis consisting of a strong bright scattering interference.

Other perspectives are now available in this field considering that a new generation ex vivo confocal laser scanning microscopy (CLSM) device is now available. This tool enables the identification of different layers of the epidermis, differentiating keratinocytes from melanocytes and permitting the visualization in detail of skin appendages including hair follicle, sebaceous and sweat glands (Hartmann et al. 2016, 376–87).

Conclusions

RCM is a developing technology that allows optical sectioning of an area of skin without the nedd for physical sectioning: it is thus ideal for dermatologists examining detailed features of a skin lesion without troubling the patient for a biopsy specimen, for selection of the optimal site when an invasive biopsy is indicated, and for dermatological surgeons determining the margins of a lesion to be excised. The development of advanced non-invasive diagnostic techniques allows tissue imaging in vivo, and recently also ex vivo, contributing to a more accurate diagnosis of skin cancers.

While histopathology remains the gold standard for the diagnosis of skin cancers, RCM is a pivotal tool that provides clues for diagnosis of pigmented and non-pigmented lesions, flat or nodular. This technique enables non-invasive exploration of skin lesions, also in difficult-to-explore sites, identifying clues of malignancy, monitoring efficacy of topical therapies, providing a support for surgical margins and recognizing different tumor subtypes.

Disclosure

The authors declare that do not have a conflict of interest and that do not have a financial rela-

tionship with any commercial entity that has an interest in the subject of this manuscript.

Contributors

All authors participated to review. All authors were involved in writing and revising the article prior to submission.

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