14 In Vivo Reflectance Confocal Microscopy for Melanoma

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In the recent years, we are observing an increase in the incidence of melanoma worldwide. Although increased awareness of melanoma has led to more efficient screening and improved understanding of melanoma tumorigenesis has guided the development of new therapeutic options, melanoma still carries significant morbidity and mortality. As many other cancers, early diagnosis and prompt surgical excision are essential. Early detection necessarily implied the development of new noninvasive diagnostic techniques. Reflectance confocal microscopy (RCM) allows dynamic imaging of the skin at cellular resolution in real time, performing optical biopsies [1–3]. Microscopic tissue elements reflect light with different refractive indices. RCM is particularly useful for imaging melanocytic lesions, such as nevi or melanoma [4, 5], because melanin has the highest refractive index of all tissue elements (n = 1.7) and acts as a naturally occurring "endogenous" contrast [4]. Several studies have demonstrated that RCM evaluation has the potential to increase sensitivity (improves melanoma detection) [6, 7] and specificity (reduces excisions of benign lesions) for melanoma [8–11].

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14.1 RCM Features According to Melanoma Subtype

The term cutaneous melanoma includes a heterogeneous subset of malignant melanocytic proliferations that differ significantly in their epidemiology, morphology, growth dynamics, and clinical behavior. Four major histological subtypes of melanoma have been described: superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM), and acral lentiginous melanoma (ALM). Less common melanoma subtypes include nevoid melanoma, desmoplastic melanoma, clear cell sarcoma, and solitary dermal melanoma.

14.1.1 Superficial Spreading Melanoma

SSM is the most common melanoma subtype (50–80% of all melanoma diagnoses) (Figs. 14.1, 14.2). The name is derived from a prolonged radial (lateral) growth phase before invasive (vertical) growth starts. Although it can develop in any anatomic location, it most likely occurs on sun-exposed areas such as the back in men and the lower limbs in women. Few melanomas can

M. Fimiani et al. (eds.), *Technology in Practical Dermatology*,
https://doi.org/10.1007/978-3-030-45351-0_14

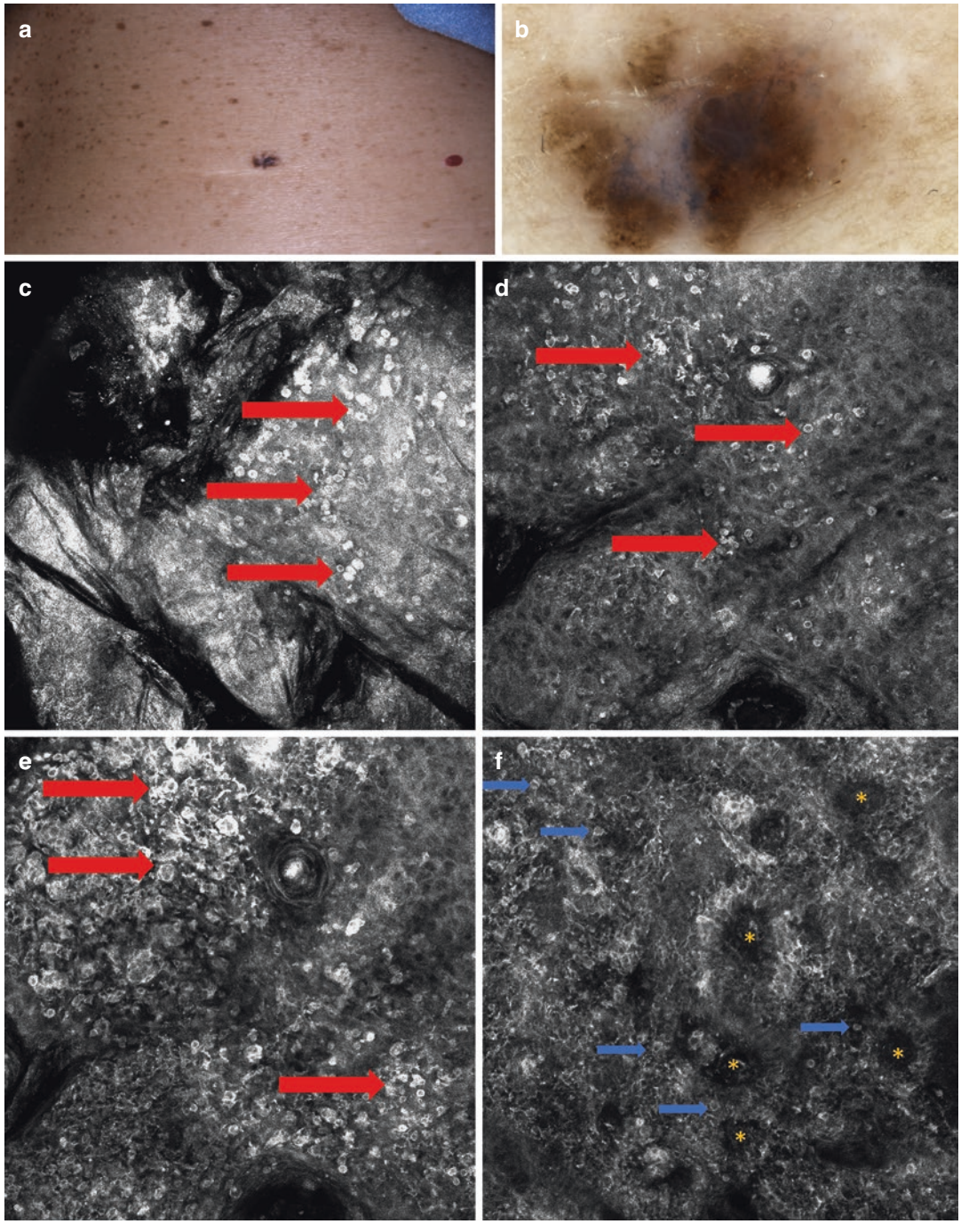

Fig. 14.1 Superficial spreading melanoma: clinical (**a**), dermoscopic (**b**), and reflectance confocal microscopy (RCM, **c**–**f**) images. RCM shows pagetoid cells (**c**, **d**, **e**, *red arrows*) in the stratum corneum (**a**), granulosum (**b**), and spinosum (**c**) of the epidermis and atypical cells (**f**, *blue arrows*) with non-edged papillae (**f**, *yellow asterisks*) at the dermo–epidermal junction that appears disarranged (**f**). (Photographer: Marco Campoli, University of Siena)

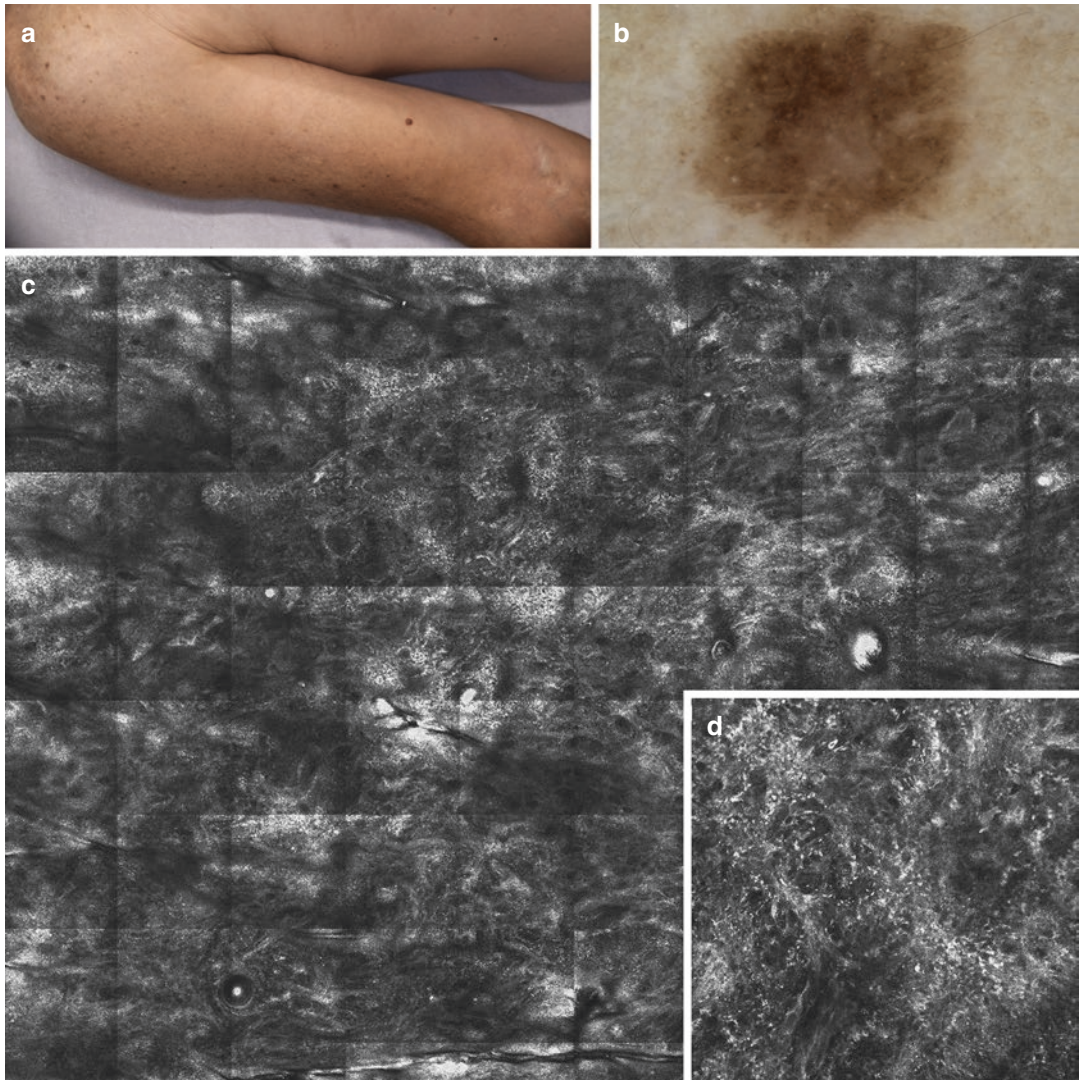

Fig. 14.2 Superficial spreading melanoma: clinical (**a**), dermoscopic (**b**), and reflectance confocal microscopy (RCM, **c** and **d**) images. RCM shows a disarranged dermal epidermal junction (**c**) due to the proliferation of bright atypical cells (**d**, detail of figure **c**). (Photographer: Marco Campoli, University of Siena)

arise from a precursor nevus and mostly occur de novo. SSM is characterized histologically by pagetoid spread and nests of the epithelioid melanocytes at the dermo–epidermal junction (DEJ). Poor circumscription with variable epidermal thickening is also common. Cytologically, melanocytes may have one or more large nuclei with an abundant cytoplasm that is often amphophilic, eosinophilic, or finely pigmented with melanin granules.

RCM criteria vary according to the examined skin layer (Table 14.1) [12]. Pagetoid spread is well visible in the epidermis (Fig. 14.1c–e). Notably, pagetoid cells appear as large hyperreflective nucleated cells in SSM. These melanocytes are mostly rounded with variably short and thick dendrites. Additionally, dendritic melanocytes could be detected, although this melanoma subtype is mainly characterized by the presence of roundish atypical melanocytes [13]. By definition, pagetoid cells under RCM have a large size (they are larger than the surrounding keratinocytes) and are scattered within the suprabasal epidermal layers without any cell connection with

Table 14.1 Reflectance confocal microscopy features of superficial spreading melanoma

Layer	Features	Description
Suprabasal epidermis	Pagetoid cells (roundish, dendritic or spindled)	Hyper-reflective (bright), large, nucleated cells (larger than the surrounding keratinocytes), typically round but may be pleomorphic
	Atypical honeycomb or atypical cobblestone pattern	Partial (poorly visible) or complete (nonvisible) loss of the normal honeycomb or cobblestone pattern caused by pagetoid spread of malignant melanocytes
DEJ	Disarranged DEJ	Irregular clod, irregular meshwork, and/or irregular ringed pattern up to complete destruction of the DEJ architecture
	Proliferation of atypical cells in single units or nests	Large cells with large nuclei and irregular size and shape
	Non-edged dermal papillae	Dermal papillae are not visible or not demarcated by a normal rim of small hyper-reflective and monomorphous keratinocytes but rather by large hyper-reflective atypical melanocytes
Upper dermis	Atypical nests	Nests composed of large and nucleated round or pleomorphic cells
	Hyper-reflective cells distributed in sheet-like structures with consequent loss of normal DEJ architecture	Hyper-reflective cells distributed in the same plane and loss of dermal papillae

the neighboring keratinocytes, giving the impression of floating cells. In situ melanomas present few or localized pagetoid cells compared with invasive tumors in which a florid pagetoid infiltration could be detected in the entire melanoma and at all epidermal layers, even in the stratum corneum [13]. The presence of atypical melanocytes can subvert the typical epidermal architecture, leading to the loss of the normal honeycomb or cobblestone pattern. Under RCM, three main tumor architectures can be detected at the DEJ: irregular ringed, irregular meshwork, and irregular clod, as well as variable combinations of all of them [13]. Furthermore, many tumors might not display any of those but rather a nonspecific pattern: dermal papillae are not clearly visible or not well demarcated by a normal rim of bright cell but rather by large reflective cells corresponding to malignant melanocytes (loss of the normal-edged dermal papillae, Figs. 14.1f and 14.2c, d). In more advanced cases, the DEJ is totally disarranged by the melanocytic proliferation. Distinct nest types can be observed according to the growth phase of the tumor: dense nests are found in early phase, whereas dense and sparse and sheet-like structures are typical of the dermal invasion [13].

14.1.2 Nodular Melanoma

NM has a fast growth rate and is associated with a higher rate of death [14]. Amelanotic or hypomelanotic presentation of NM is particularly challenging [15] and can be mistaken for benign tumors. At RCM (Table 14.2), NM reveals a thinned epidermis (epidermal consumption) with the typical epidermal architecture still recognizable and few pagetoid cells [16]. The DEJ is completely disrupted, and the dermal compartment is filled with a solid proliferation of melanocytes with variable shape and size arranged as single cells or clustered. The so-called sheet-like structures that represent a proliferation of dyscohesive atypical melanocytes with prominent nuclei are frequent in NM. Remarkably, melanocytic nests with cerebriform appearance (cerebriform nests) are specific of NM and melanoma skin metastasis. Those nests show up as dark hyporeflective clusters of melanocytes that are outlined by brighter collagen fibers. Enlarged and tortuous vessels can also be frequently found. Tumor clusters are commonly found in proximity to these newly formed vessels [12].

Table 14.2 Reflectance confocal microscopy features of nodular melanoma

Layer	Features	Description
Suprabasal epidermis	Few or no pagetoid cells	Pure NM have fewer pagetoid cells than SSM
	Thin epidermis with normal epidermal architecture	Preserved honeycomb or cobblestone pattern (differing from SSM that exhibit distortion of the normal epidermal pattern)
DEJ	No visible dermal papillae	Substituted by a proliferation of atypical melanocytes often arranged in sheets
Upper dermis	Cerebriform nests	Aggregation of small compact cells with cerebriform appearance
	Enlarged vessels	

14.1.3 Lentigo Maligna and Lentigo Maligna Melanoma

Lentigo maligna (LM) presents as a slowly progressive pigmented macule on sun-exposed areas, most commonly on the face (Figs. 14.3a and 14.4a) and neck, and LMM is its invasive counterpart. Its clinical diagnosis is often challenging because it shows overlapping features with benign lesions. As it is often large and located on esthetic and functional areas, noninvasive imaging techniques such as dermoscopy and RCM are of great interest for its diagnosis. Dermoscopy has improved the diagnosis of LM/LMM, but they remain a challenge owing to overlapping features with solar lentigo, pigmented actinic keratosis, and lichenoid keratosis (Figs. 14.3b and 14.4b) [17, 18]. Histopathology could also be of difficult interpretation due to atypical melanocytic proliferation on sun-damaged skin [19]. RCM has proved to be helpful to enhance diagnosis of LM/LMM. Features of LM/LMM using RCM are well described [20–25] (Table 14.3). The earliest histopathological finding in LM is the proliferation of atypical melanocytes at the DEJ [26] that correlate with large hyper-reflective polymorphic cells under RCM (Figs. 14.3c and 14.4c, d). Numerous large pagetoid cells and consequent epidermal disarray are subsequently found. In LM/LMM, atypical melanocytes are more often dendritic than roundish and are typically located around and inside hair follicles (Figs. 14.3c and 14.4c, d). In some cases, only numerous hyper-reflective dendrites are visible, and not cell bodies and these hyper-reflective long dendrites form "medusa head-like structures" (Fig. 14.3c) around hair follicles. Advanced cases show large nucleated cells organized in nests in the upper dermis [12].

14.1.4 Acral Lentiginous Melanoma

The clinical and histological diagnosis of ALM may be very difficult, especially in the early phase of growth. Palms and soles are rarely studied by RCM because of the increased thickness of the epidermis that hampers the visualization of deeper layers. However, RCM can also be useful in acral site because early ALM is characterized by a lentiginous spread [27], and the epidermal thickness of acral areas is variable.

Pagetoid cells are the predominant clue for suspecting ALM by RCM [28]. The pagetoid spread of melanocytes also determines a disarrangement of the normal honeycomb pattern of the epidermis. Noteworthy in case of a thick stratum corneum, a skin scraping could allow to identify pagetoid cells in the epidermis that are at first not visible. It should be noticed that a proliferation of solitary arranged melanocytes can also be detected within the epidermis of melanocytic acral nevus with intra-epidermal ascent (MANIACs) [29]. However, pagetoid cells in these cases are monomorphous [28].

A characteristic feature of ALMs is the infiltration of sweat duct structures by atypical bright cells that correspond to a melanocytic extension along adnexal structures in histology. Nevi show more rarely a melanocytic proliferation around sweet duct structures and in these cases, melanocytes are mainly organized in nests [28, 30]. Another peculiar feature of ALM is the higher presence of granular dust-like hyper-reflective particles in the epidermis compared to

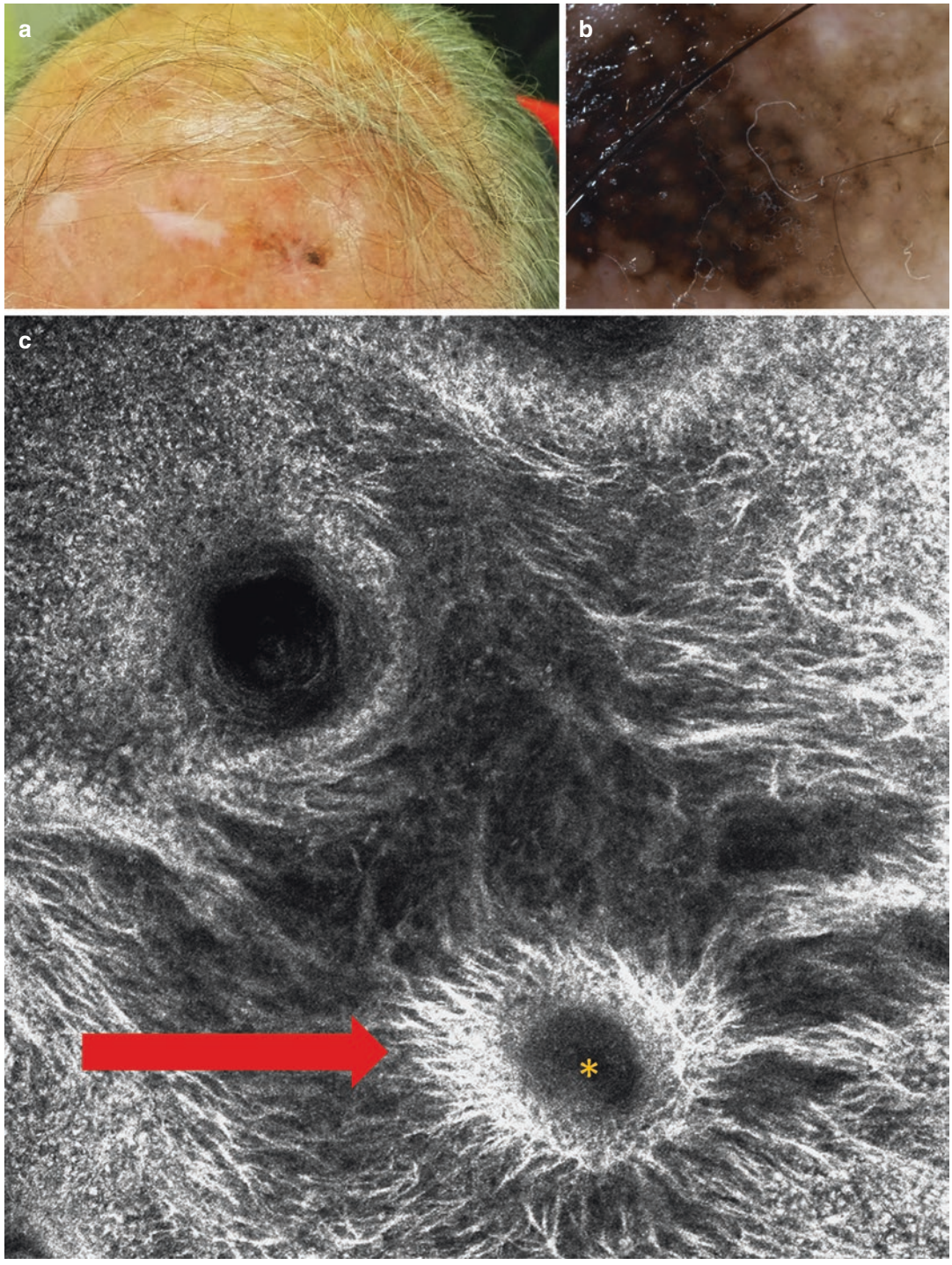

Fig. 14.3 Lentigo maligna: clinical (**a**), dermoscopic (**b**), and reflectance confocal microscopy (RCM, **c**) images. RCM shows a proliferation of atypical dendritic melanocytes in the epidermis (*red arrow*) that mainly infiltrate hair follicles (*yellow asterisk*) with the so-called medusa head-like structures. (Photographer: Elisa Cinotti, University of Siena)

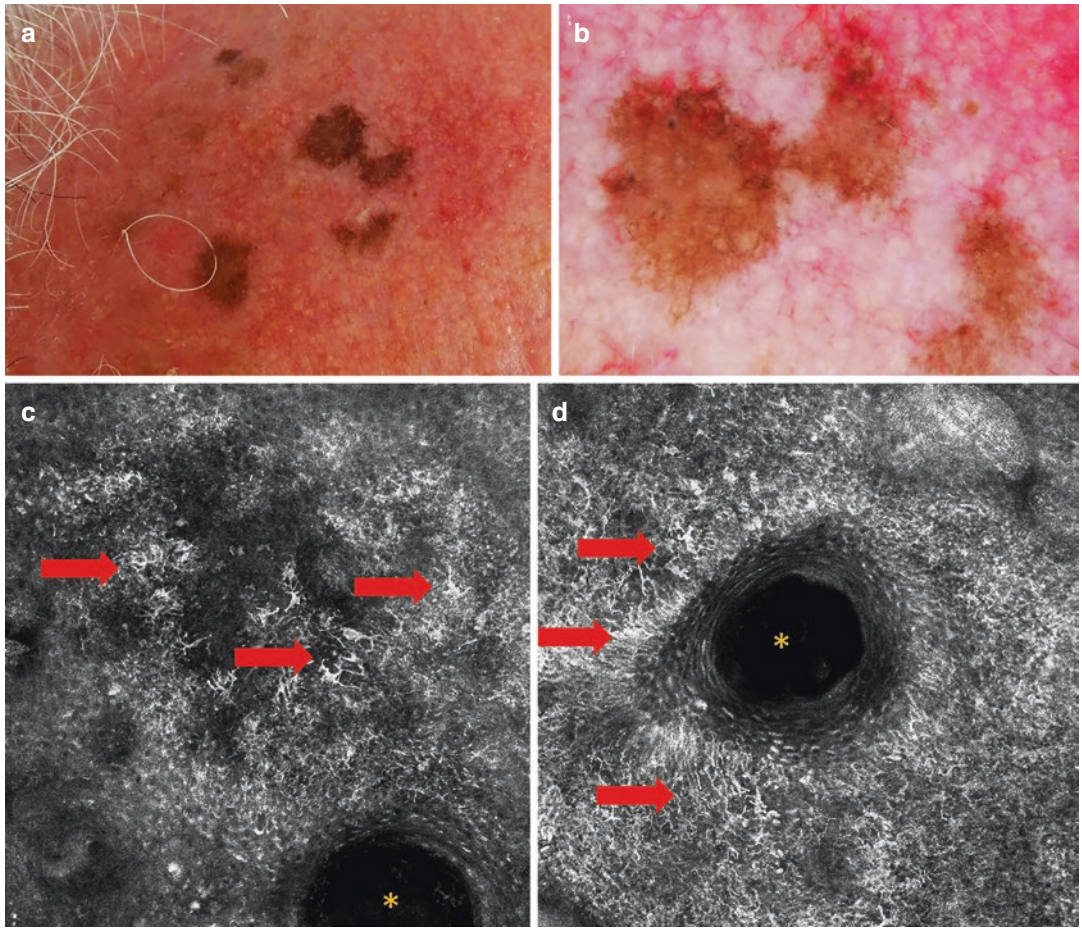

Fig. 14.4 Lentigo maligna: clinical (**a**), dermoscopic (**b**), and reflectance confocal microscopy (RCM, **c**) images. RCM shows a proliferation of atypical melanocytes in the epidermis (*red arrow*) that have a tropism for hair follicles (yellow asterisk). (Photographer: Elisa Cinotti, University of Siena)

Table 14.3 Reflectance confocal microscopy features of lentigo maligna/lentigo maligna melanoma

Layer	Features	Description
Suprabasal epidermis	Large dendritic and/or roundish hyper-reflective pagetoid cells	
	Epidermal disarray	No recognizable honeycomb or cobblestone pattern
DEJ	Non-edged papillae	Loss of normal rim of bright keratinocytes around the dermal papillae due to atypical cell proliferation
	Follicular localization of atypical cells	Large dendritic and/or roundish cells around and inside hair follicles
	Medusa head-like structures	Elongated bundles, composed of dendritic atypical cells, extending from the hair follicles
Upper dermis	Isolated large nucleated cells in the dermal papillae	
	Dermal nests	Aggregation of atypical melanocytes

nevi, possibly corresponding to free pigment. Free pigment is randomly distributed in ALMs and tends to be arranged in columns in the furrows in nevi [30].

In all ALMs with a visible dermis, sheets and/or nests of large atypical cells are usually seen, whereas these features are never found in nevi [28]. However, ALM diagnosis cannot be excluded in the absence of RCM signs because early ALM can present only subtle atypia such as slight proliferation of atypical melanocytes at the DEJ that may not be identified under RCM [28].

14.1.5 Desmoplastic Melanoma

Desmoplastic melanoma is a rare melanoma subtype that most commonly occurs on chronically sun-exposed areas of elderly patients. Misdiagnosis is common as it is often amelanotic and may mimic a scar or benign cutaneous tumor, such as dermatofibroma [31]. This melanoma is characterized by bundles of spindle-shaped melanocytes admixed with dense collagen and patchy lymphoid infiltrate in the dermis. These cells have a fibroblast-like appearance but hyperchromatic and bizarre nuclei are visible. The junctional component is minimal.

RCM features that may suggest the diagnosis of desmoplastic melanoma in the upper dermis are spindle melanocytes (elongated hyper-reflective large and often nucleated cells) and inflammation (presence of small hyper-reflective roundish cells).

14.1.6 Amelanotic Melanoma

The diagnosis of amelanotic melanoma is clinically challenging (Fig. 14.5a). Confusions with benign skin lesions or nonmelanoma skin cancer are potential pitfalls. Dermoscopic evaluation is useful to detect subtle signs of amelanotic melanoma, such as the presence of dotted vessels, linear irregular vessels, and milky red areas (Fig. 14.5b) [32, 33]. Amelanotic lesions are a major indication for RCM, as even small amounts of melanin can be seen with RCM [20]. RCM findings in amelanotic melanoma are similar to pigmented melanoma and include the proliferation of polymorphic large cells in the epidermis, DEJ (Fig. 14.5c, d) and dermis, loss of normal honeycomb pattern in the suprabasal epidermis, and DEJ disarray with irregular dermal papillae [34]. Pagetoid cells may appear dendritic or roundish. Unlike the hyper-reflective atypical cells of pigmented melanomas, melanocytes of amelanotic or hypomelanotic melanomas are hypo-reflective because of the lack of melanin and may appear as dark holes in the epidermis [35].

14.1.7 RCM Diagnostic Algorithms for Melanoma Diagnosis

Five main scoring systems and algorithms have been developed for the diagnosis of melanoma using RCM [1, 20, 36–41].

- The Modena algorithm: Pellacani et al. identified six RCM criteria that independently correlate with the diagnosis of melanoma and may be used to differentiate melanomas from nevi [42, 43]. The scoring algorithm is composed of two major criteria (two points each) and four minor criteria (one point each). A score greater than or equal to 3 is strongly associated with the diagnosis of melanoma (97.3% sensitivity and 72.3% specificity).
  The major criteria (+2 points per feature) are:
  - Non-edged dermal papillae
  - Atypical cells at the DEJ

  The minor criteria (+1 point per feature) are:
  - Roundish pagetoid cells
  - Pagetoid cells widespread throughout the lesion
  - Cerebriform clusters in the papillary dermis
  - Isolated nucleated cells within dermal papilla
- The Barcelona algorithm: Segura et al. [37] developed a two-step method for differentiating melanocytic from nonmelanocytic lesions and melanoma from nevi using RCM.

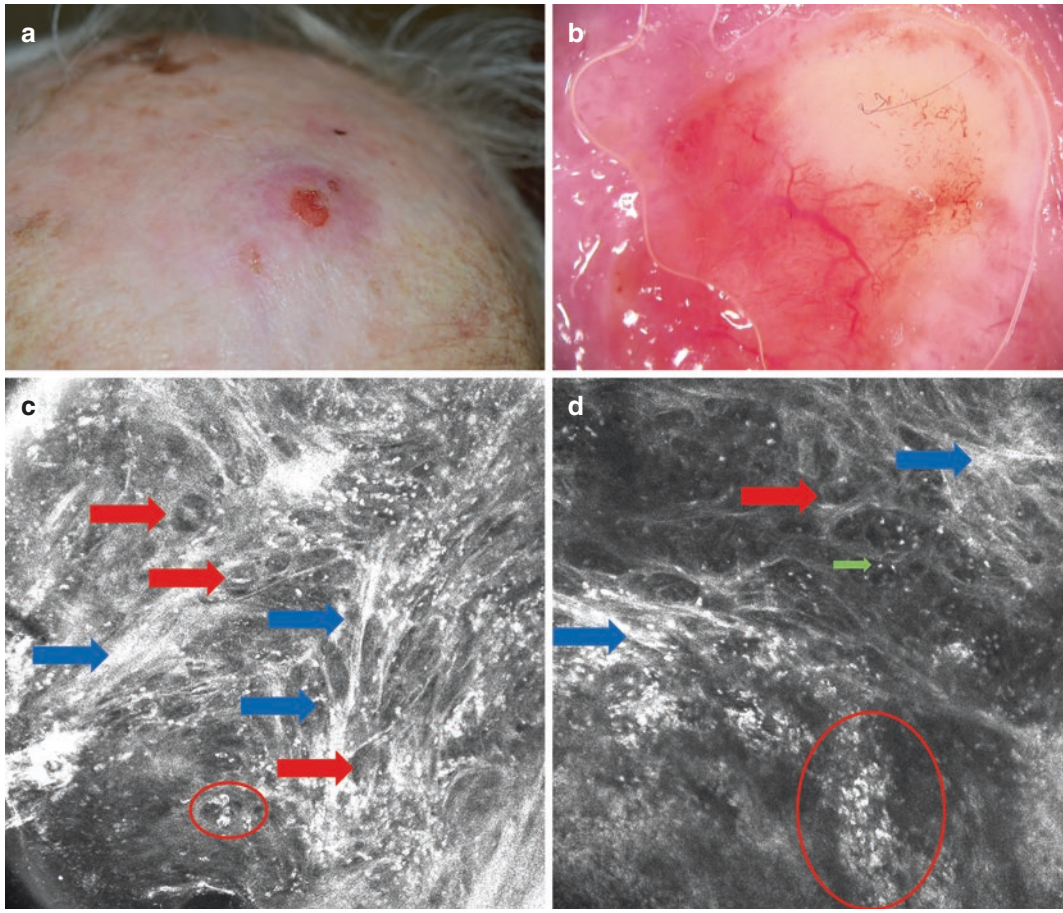

Fig. 14.5 Amelanotic nodular melanoma: clinical (**a**), dermoscopic (**b**), and reflectance confocal microscopy (RCM, **c**, **d**) images. RCM shows in the dermis nests of hypo-reflecting (*red arrows*) and hyper-reflective (*red circle*) atypical cells surrounded by collagen fibers (*blue arrows*) and isolated small hyper-reflective cells corresponding to inflammatory cells (*green arrow*). (Photographer: Elisa Cinotti, University of Siena)

The two steps are:

- Step 1: to determine if the lesion is melanocytic or nonmelanocytic. A melanocytic lesion is suspected based on the presence of at least one of four RCM features. Features of a melanocytic lesion include cobblestone pattern, pagetoid spread, meshwork appearance at DEJ and presence of dermal clusters of cells or dermal nests.
- Step 2: to determine if the lesion is a nevus or a melanoma using a scoring system. Benign features (absence of basal cell atypia and edged papillae) are given a score of −1, while malignant features (roundish pagetoid cells and atypical dermal nucleated cells) a score of +1. Lesions with a score greater than or equal to −1 have a sensitivity and specificity, respectively, of 86.1 and 95.3%, for melanoma. However, with this threshold there were five false-negative results in their study, including four in situ and one SSM, indicating the possible limitation of the algorithm in thin melanoma. A score greater than or equal to −2 had an increased sensitivity of 100% but specificity reduced to 57%. The authors calculated that, despite a lower specificity, half of biopsies could be avoided, without missing a melanoma.

- Guitera et al. [36] described a two-step method to diagnose melanoma and basal cell carcinoma (BCC). Independently significant RCM features were identified to establish the following criteria in a two-step algorithm. The first step is to determine if the lesion is a BCC by the analysis of positive and negative features.
- The second step is to determine if the lesion is a melanoma using the Modena algorithm.
- Borsari et al. [41] recently proposed a diagnostic score for melanoma in situ (MIS) combining dermoscopic and RCM features. Dermoscopic finding of atypical pigment network and regression gives 1 point each. On RCM, the observation of pagetoid cells gives 1 point and cytologic atypia 1 point if it is focal and 1 points if it is widespread. The presence of dense nests and melanophages was found to be protective of MIS and given −1 point each.
- Guitera et al. [20] developed the LM score to assist in differentiating LM from equivocal pigmented macules of the face. Six features that independently correlate with the diagnosis of LM were identified. The score consists of two major and four minor criteria. With a score more than or equal to 2 points, a sensitivity and specificity of 85 and 76%, respectively (odds ratio: 18.6; 95% CI: 9.3–37.1), were achieved for LM diagnosis. The major criteria are: non-edged dermal papillae (+2) and large round pagetoid cells (+2). The minor criteria are: nucleated cells in the dermal papillae (+1), atypical cells at the DEJ (+1), adnexal spread of atypical cells (+1), and broadened honeycomb pattern (−1).

14.1.8 Clinical Application of RCM for LM/LMM

Among all melanoma subtypes, RCM is most applicable to the management of LM and LMM due to the radial growth of this neoplasia and the limited depth penetration of RCM. Moreover, noninvasive diagnosis and identification of surgical margins are particularly important for LM/LMM because this melanoma is mainly located on esthetic and functional areas such as the face, and it may have subclinical extension.

14.1.9 Guide for Biopsies

LM/LMM often has a large surface, and a single biopsy could miss the area with the typical histological features. RCM may be used to assist in targeting the area for incisional biopsy to the most suspicious area for a correct diagnosis and for assessing the level of invasion [44].

14.1.10 Preoperative Mapping

LM/LMM has the highest risk of residual disease and highest recurrence rate of all melanoma subtypes after surgical excision [45]. RCM may allow the identification of subclinical cancer invasion [25, 46]. Presurgical mapping of the suspicious area helps surgeons planning the surgery and the eventual reconstruction. It has been used successfully intraoperatively to achieve negative surgical margins in a case of standard excision [47] and also in combination with the staged excision spaghetti technique [48, 49]. Margin delineation of LM and LMM with the handheld RCM (HRCM) could be limited by the lack of precise orientation during imaging and the small field of view in the absence of mosaics. Video mosaicking of HRCM images is a novel technique developed to overcome this limitation [50].

14.1.11 Monitoring of Nonsurgical Therapies

RCM can be used to assess response to treatment of LM/LMM and allow detection of treatment failure with better diagnostic accuracy than dermoscopy, especially after nonsurgical treatments (e.g., radiation therapy or topical imiquimod) [51, 52]. An advantage of RCM is that it enables the clinician to follow difficult cases with serial noninvasive “virtual biopsies” of the skin.

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