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Seminars in Cutaneous Medicine and Surgery

Reflectance Confocal Microscopy—State-of-Art and Research Overview

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Reflectance confocal microscopy (RCM) enables in vivo imaging of human skin at a quasi histologic resolution. The black-and-white RCM images show horizontal sections of the skin, at a maximum depth of 350 μm. To date, the RCM features of a significant number of skin conditions have been described. The main focus of the research community investigating RCM, however, lies on describing and diagnosing melanocytic skin lesions. Taking into account all RCM studies dealing with diagnostic accuracy in melanocytic skin lesions, sensitivity and specificity of approximately 90% and 86% could be found. Improvement of diagnostic accuracy, improved assessment of dermoscopic-histologic correlation, in vivo biopsy side selection, surgical margin assessment, and response control of conservative therapies in skin diseases are some of the major advantages of this novel imaging method. Additionally, RCM holds inherent potential for teledermatologic application and automated image analyzing. This article describes morphologic features of diverse skin lesions and features of "normal skin," summarizes diagnostic advances of RCM, compares studies dealing with diagnostic applicability, and discusses further research goals of this exciting new imaging technique.

Semin Cutan Med Surg 28:172-179

The attempt to improve accuracy of dermatologic diagnoses, especially for melanocytic skin lesions, has led to the development of noninvasive imaging tools, such as dermoscopy, optical coherent tomography, magnetic resonance imaging, and high-frequency ultrasound.[1] Although most of these new imaging systems are mainly applied in experimental settings for research purposes, dermoscopy was successfully introduced in routine skin tumor screening and has the widest clinical use. Dermoscopy revealed features underneath the skin surface previously not visible to the naked eye and thereby enhanced the number of diagnostically useful criteria. However, despite several achievements, the relatively low magnification in routinely used instruments and the limited scope of observable structures restrict the usefulness and diagnostic applicability of the method. Among novel imaging tools, reflectance confocal microscopy (RCM) stands out because of its high resolution. The technique was first described for skin imaging by Rajadhyaksha et al in 1995.[2] In recent years, major improvements in image quality and clinical applicability have been made.

RCM allows noninvasive examination of native skin in real-time at a nearly histologic resolution. With RCM, it is possible to image skin in vivo and freshly biopsied skin without fixing, sectioning, and staining, which would be necessary for the preparation of conventional histologic slides. The resolution enables imaging of nuclear, cellular, and tissue architecture of epidermis and the underlying structures, including connective tissue, inflammatory infiltrates, tumor cells, capillaries, and even circulating blood cells.[2]

The reflectance confocal microscope emits a near-infrared, coherent laser beam by which the human skin is illuminated. As the laser beam passes through the upper skin layers, it is partially backscattered due to the natural refractive index of microanatomical structures. This backscattered light has to pass through a narrow pinhole, which guarantees that only light reflected from structures "in focus" is detected; light from elsewhere is blocked. After passing the pinhole, the beam is diverted by a semireflective mirror system and, finally, directed to a detector. The obtained data are processed and visualized by special software on a computer screen (Fig. 1).

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 1085-5629/09/$-see front matter
doi:10.1016/j.sder.2009.06.004

Figure 1 The first commercially available in vivo reflectance confocal microscope, Vivascope 1000.

In contrast to histologic slides, which show colored vertical sections, the black-and-white RCM images correspond to horizontal (en face) sections at a selected depth within the skin. RCM reveals skin changes at a cellular level. Contrast is provided by different refraction indexes of distinctive intracellular structures. Highly refractile structures, such as melanin, intercellular connections, or cytoplasmic granules, appear bright, while less refractile structures, such as cell nuclei, appear dark in RCM images.

Briefly, commercially available reflectance microscopes, developed for the examination of human skin, use a near-infrared laser beam at 830 nm, operating at a power of less than 35 mW. The low power of the laser guarantees that no tissue damage occurs. The maximum depth of imaging is 350 μm, dependent on the examined tissue. The relative anatomic imaging depth is also dependent on skin thickness, allowing visualization of the epidermis and the superficial dermis. The 30-fold water-immersion objective lens offers a 160-800 μm field-of-view, providing a lateral spatial resolution of about 1.0 μm and an axial spatial resolution of 3-5 μm. As the axial resolution is less than the depth of a single cell layer, it is possible to visualize intracellular details.[3]

Images can be scanned horizontally, with small quadratic fields-of-view forming a square mosaic of contiguous 500 × 500 μm images (Fig. 2C), the RCM mosaic. This RCM mosaic represents an overview image with 5-fold magnification (Fig. 2B). Moreover, by moving the focus plane into or out of the tissue (along the z-axis), a stack of images can be generated. The imaging procedure for 1 single lesion requires approximately 5-15 minutes.

This article describes morphologic features of diverse skin lesions and features of "normal skin," summarizes diagnostic advantages of RCM, compares studies dealing with diagnostic applicability, and discusses further research goals of this exciting new imaging technique.

RCM Features of Normal Skin

RCM of the stratum corneum reveals large bright, anucleated cells with polygonal shapes (diameter, 10-30 μm) with dark outlines. The brightness of the corneocytes is caused by the difference in the refractive indexes at the interface between the immersion medium and the stratum corneum, which results in a large amount of back-scattered light. The corneocytes form islands separated by skin folds, which appear very dark.[4] The subjacent stratum granulosum is located 15-20 μm below the skin surface. It typically presents large polygonal cells (diameter, 10-25 μm) with bright, reflectant granular cytoplasm and a central dark nucleus.

The stratum spinosum is located 20-50 μm below the skin surface. It consists of small cubical cells (diameter, 15-25 μm) with bright cellular borders due to occasional melanin granules and intercellular connections (structures with high refractive index). Cells of the stratum spinosum and stratum granulosum form a cohesive honeycomb pattern with bright granular cytoplasm and dark oval to round nuclei in the center (Fig. 3).

The basal layer is located approximately 40-100 μm below the skin surface. Basal cells have a diameter of about 7-12 μm. They typically appear as solitary bright, round to oval shapes in RCM images. The nuclei present as dark halos. Basal cells form a cobblestone pattern. Pigmented keratinocytes, as well as melanocytes, appear very bright in RCM, due to the high refractive index of melanin. In normal skin, it is difficult to clearly distinguish melanocytes and pigmented basal keratinocytes, because melanocytes rarely show branching outlines that may correspond to their dendrites and appear as round to oval structures.[5] Basal cells may form bright rings around the dermal papillae, which appear as dark holes in the epidermis ("edged papillae").[4,5] The papillary dermis can be found 50-150 μm below the skin surface. The dermal papillae appear as dark, round to oval, homogeneous areas with central vascular loops, surrounded by a corona of bright basal cells (Fig. 4). In real-time RCM imaging, the movements of individual blood cells inside the papillary vessels can be observed. The papillary vessels appear darker than the surrounding stroma. They are surrounded by a network of collagen fibers (1-5 μm) and various cell populations. Among these, melanophages are easily recognized due to their high refractivity. Melanophages can be observed near blood vessels in the upper dermis. They appear as large, intensely reflecting cells with irregular shapes, without nuclei.[5] The superficial portion of the reticular dermis can only be observed in thin-skin areas and shows collagen bundles (5-25 μm), which have a bright periphery and a dark center.

Appendageal structures, such as hair follicles and eccrine glands, are also visible in RCM. Eccrine sweat ducts appear as bright, oval to round structures with a dark center that spiral through the epidermis and dermis. Hair shafts with pilosebaceous units appear as bright, circular structures with elliptic elongated cells at the circumference and a central refractive long-hair shaft.

It has to be considered, however, that the confocal features described above may vary depending on various variables. "Normal skin" has a great variability in thickness and other characteristics according to a person's age, sex, race, and the body area.[6]

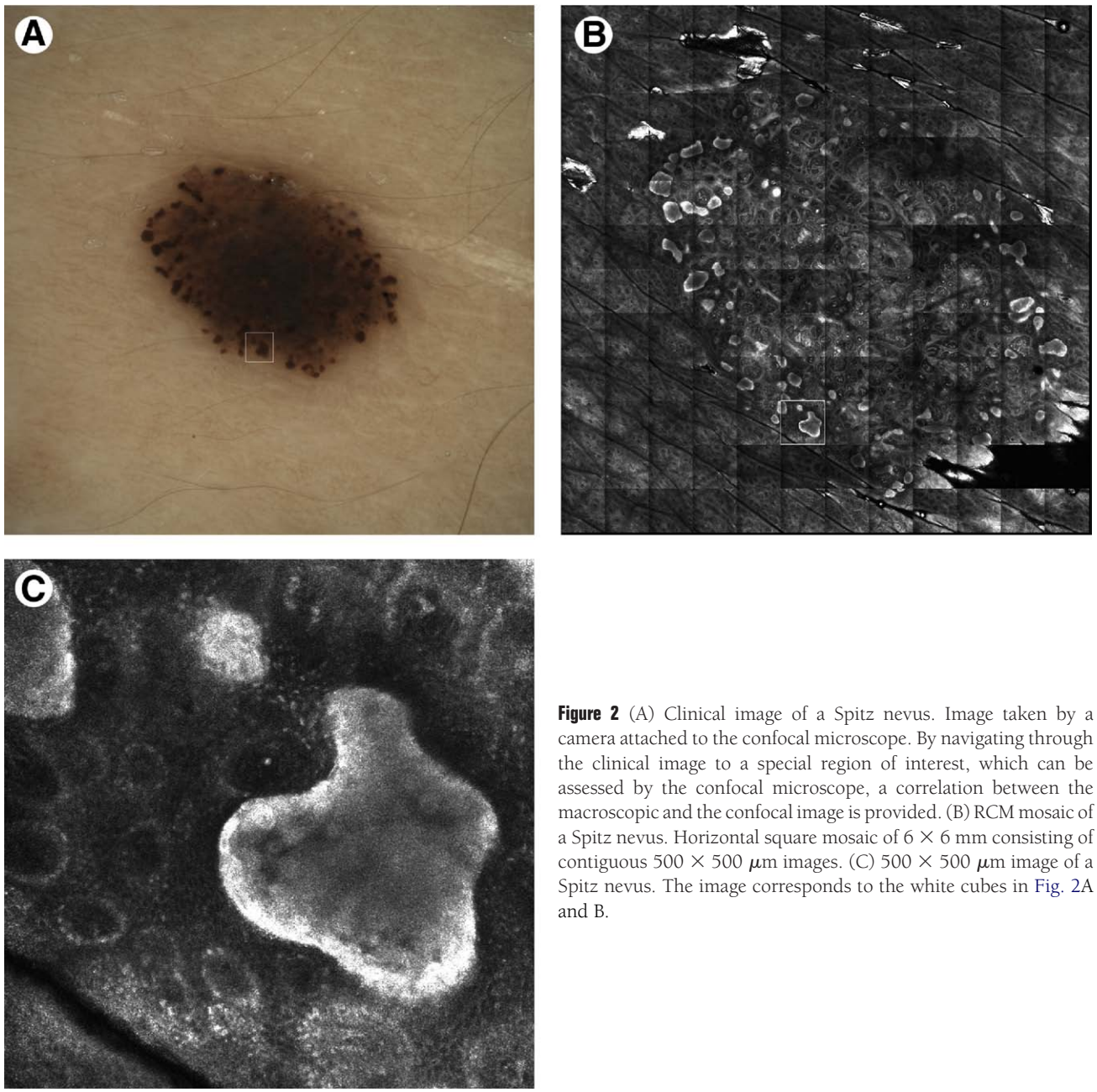

Figure 2 (A) Clinical image of a Spitz nevus. Image taken by a camera attached to the confocal microscope. By navigating through the clinical image to a special region of interest, which can be assessed by the confocal microscope, a correlation between the macroscopic and the confocal image is provided. (B) RCM mosaic of a Spitz nevus. Horizontal square mosaic of 6 × 6 mm consisting of contiguous 500 × 500 μm images. (C) 500 × 500 μm image of a Spitz nevus. The image corresponds to the white cubes in Fig. 2A and B.

RCM Features of Melanocytic Skin Lesions

The current RCM research focuses primarily on diagnosis and differentiation of benign and malignant melanocytic lesions. Because melanin provides strong contrast in RCM, melanocytes can be easily assessed,[7] enabling an enhanced diagnosis of melanocytic lesions, detection of local recurrences after surgical excision, and in vivo tumor follow-up.

A systematic examination and comparison of RCM features of common nevi was first performed by our study group in 2008.[5] Morphologic aspects of dermoscopically observed reticular, globular, and homogeneous patterns in benign nevi were described.

In common nevi, melanocytes normally present as small, round to oval, bright and monomorphous cells. Rarely, nevus cells show short and fine dendrites. The honeycomb pattern of keratinocytes in the epidermis is preserved, and the cell borders of adjacent keratinocytes can be easily detected.

In reticular nevi, uniformly distributed dermal papillae, which were circumscribed by a rim of refractive cells ("edged papillae"), were found (Fig. 5). Only dark dermal papillae without reflective structures were observed in this nevus subtype.[5]

In globular nevi, melanocytes were found to be arranged in well-defined highly reflective nests at the dermo-epidermal junction or clustered within the papillary dermis (Fig. 6). Melanophages could be visualized as large, intense reflecting cells with ill-defined cell borders and bright, grainy cyto-

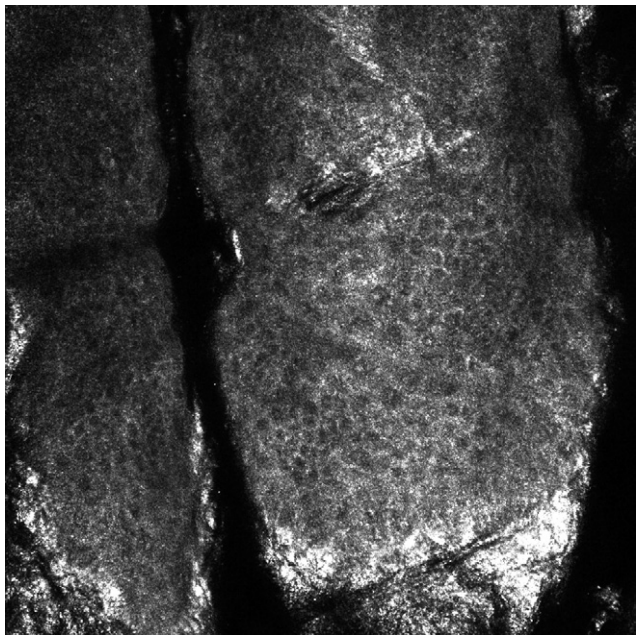

Figure 3 Normal skin. 500 × 500 μm RCM image showing a regular honeycomb pattern composed of the keratinocytes of the stratum corneum. The dark areas correspond to the skin folds.

plasm usually located around or near vessels of the superficial dermis. Well-defined edged papillae could not be disclosed. Dermal papillae showed a higher content of reflective melanophages and melanocytic nests (white papillae).[6]

Homogeneous nevi showed overlapping features of both nevus subtypes described above. They often showed dark papillae in vicinity to white papillae filled with refractive melanophages and melanocytic nests.[5]

Melanomas (MMs) typically show solitary, polymorphic, and irregularly shaped tumor cells, which frequently form dendrite-like structures with a complex branching pattern. Atypical cells may be found ascending in several layers of the epidermis, representing pagetoid spread (Fig. 7). Architectural disarray (ie, a disruption or loss of the normal honeycomb and cobblestone pattern of keratinocytes) can also be observed, with absent or poorly defined keratinocyte cell borders. In general, the skin layers show an architectural disarray and single cells predominate over cell nests. Dermal papillae appear irregularly sized and shaped. As described above, basal cells commonly form bright rings around the dermal papillae, which appear as dark holes in the epidermis. These papillae are described as "edged." In MMs, however, frequently "non-edged" papillae, which are not sharply demarcated, can be observed. Pigment is distributed unevenly throughout the lesion. In invasive MMs, however, the limited maximum image depth may hinder analysis of deeper structures. Segura et al described in a recently published study[8] that a detection of dermal cell clusters in nodular MMs and in superficial spreading MMs with nodular component was possible in 70% of cases, whereas nodular MMs lacked "typical"

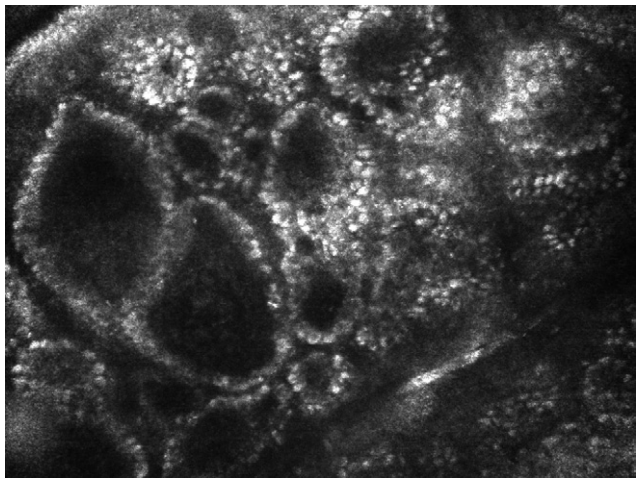

Figure 5 Reticular nevus. This nevus subtype typically shows uniformly distributed dermal papillae, which are circumscribed by a rim of refractive cells ("edged papillae"). Dermal papillae appear dark without refractive structures.

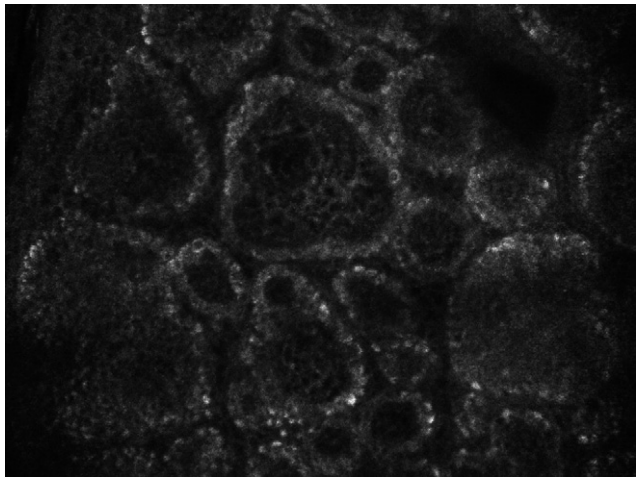

Figure 4 Normal skin. 500 × 500 RCM image at the level of the dermo-epidermal junction. Basal cells typically appear as solitary, bright to oval shapes. Dermal papillae appear as dark holes.

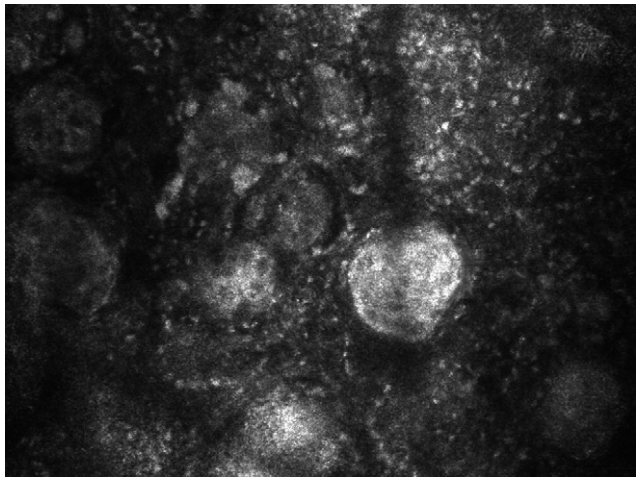

Figure 6 Globular nevus. In this nevus subtype, dermal papillae show a higher content of refractive melanophages and melanocytic nests ("white papillae").

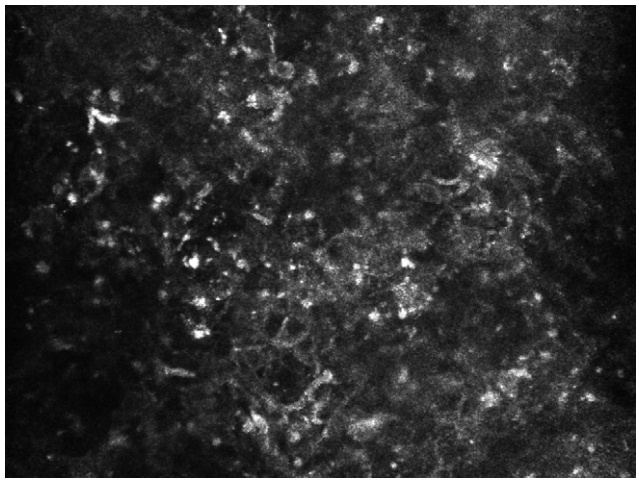

Figure 7 Melanoma (MM). MMs typically show solitary, polymorphic irregularly shaped tumor cells. Atypical cells may be found ascending in several layers of the epidermis, representing pagetoid spread.

MM features, such as architectural disarray and pagetoid infiltration in the epidermis. Of note, these observations, together with the results of stem cell research, have stimulated a reconsideration of the conventional concept of MM development.[9]

Even amelanotic MMs can be recognized by RCM. The presence of melanosomes in the cytoplasm, which acts as an endogenous source of contrast, and probably the presence of some melanin in premelanosomes provide contrast to visualize lesional melanocytes.[10,11]

In a multivariate analysis, Pellacani and coworkers[12] described 6 criteria as independently correlated with diagnosis of MM. Two major criteria were described: (i) atypical cells at the dermo-epidermal junction and (ii) non-edged dermal papillae. Four minor criteria were found: (i) roundish cells in the superficial layers spreading upward in a pagetoid spread; (ii) widespread pagetoid infiltration throughout the epidermis; (iii) cerebriform cell clusters in the papillary dermis; and (iv) isolated nucleated cells within dermal papillae. Interestingly, as described in a more recent study[13] identification of pagetoid infiltration was found to represent the most relevant discriminant feature. Tumors showing roundish pagetoid cells had a 15 times greater risk of being malignant. By contrast, normal-appearing epidermal architecture seemed to be specific for benign lesions.

In atypical nevi, intermediate characteristics between monomorphous features observed in benign nevi and polymorphous features observed in MMs can be found, depending on the degree of cellular atypia. In atypical nevi, the cell population is more heterogeneous in size, shape, and refractivity. However, cells tend to be round or oval as in common nevi. Cell nests are less defined and may fuse forming broad fields of nevus cells. Keratinocyte cell borders show focal absence in the epidermis. Atypia correlates with attenuated brightness in melanocytes, with isolated large bright epithelioid cells with peripheral nuclei.

A diagnostic pitfall in RCM is the diagnosis of Spitz nevi, which can often be misdiagnosed as MM and vice versa. Spitz nevi often show pagetoid infiltration, architectural disarray, and atypical cells.[14] Pellacani and coworkers[15] recently described correlation of RCM features with dermoscopic and histopathologic features in Spitz nevi. Features of 40 Spitz nevi were compared with those of 40 MM and 40 Clark nevi, respectively. The presence of sharp border cutoff, junctional nests, and melanophages (as mostly found in Spitz nevi) were described as the most characteristic features for differentiation of MM from Spitz nevi. Nevertheless, often Spitz nevi remain undistinguishable from MM due to overlapping features and the impossibility of observing lesions in deep vertical depth.[16]

RCM Features of Nonmelanocytic Skin Lesions

Diagnosis and follow-up of nonmelanoma skin cancers is another area of interest in current RCM research, because these tumors are very common.

Basal cell carcinomas typically show a “streaming” pattern of large, elongated cells with dark nuclei. In deeper layers, bright collagen-fiber bundles and an increase in number and diameter of blood vessels with loss of vascular architecture can be observed.[17]

RCM also allows differentiation between actinic keratoses and surrounding sun-damaged skin. Actinic keratoses typically show an inhomogeneous, irregular stratum corneum, and distorted architecture of the honeycomb pattern of keratinocytes of the stratum granulosum and stratum spinosum. Moreover, architectural disarray and pleomorphism of the epidermal layers can be observed.[18,19]

Squamous cell carcinomas can only be analyzed in vivo in the case of mild hyperkeratosis. Moreover, the dermal component (tumor aggregates, increased number of vessels, solar elastosis) may be hidden to the observer, due to minimal light penetration to the deeper layers. The superficial layers show similar changes to those observed in actinic keratosis, although more severe.[19]

RCM—Research Overview

RCM is a relatively young field of research. Nevertheless, major progress has been made in the description and in vivo diagnosis of melanocytic skin tumors in the past few years. To date, the RCM features of a significant number of skin conditions have been objectively characterized. The main focus of the research community investigating RCM, however, lies on describing and diagnosing melanocytic skin lesions. In preliminary studies, numerous morphologic RCM features of melanocytic lesions have been described and histopathological as well as dermoscopic correlates of confocal structures have been elucidated.[20-23] To create a standardized RCM terminology, a Consensus on Confocal Reflectance Imaging was held in 2007.[4] Furthermore, in various studies the diagnostic applicability of RCM in melanocytic skin tumors has been evaluated.

The diagnostic applicability of RCM in melanocytic skin tumors, determined by evaluating sensitivity, specificity, as well as positive- and negative-predictive value (PPV, NPV), was first described by our research group in 2005.[24] The study sample consisted of 117 melanocytic skin lesions (90 benign nevi, of which 30 were verified by histopathology; and 27 MMs, all verified by histopathology). Five observers without previous experience in RCM evaluated the confocal images. They received a standardized instruction about RCM features of melanocytic lesions[25,26] for 30 minutes. The observers were blinded to the clinical, dermoscopic, and histopathological diagnoses of the melanocytic lesions. Overall, a sensitivity of 88.15% and a specificity of 97.60% (PPV 90%, NPV 96.94%) were achieved by the 5 observers. Logistic regression analysis of the combination of all features found that melanocyte cytomorphology, melanocytic architecture, and keratinocyte cell borders were highly specific and sensitive features.

In a second study, we extended our study sample to nonmelanocytic skin tumors, including 15 cases of basal cell carcinoma and 30 cases of seborrheic keratoses.[27] Diagnosis of MM and all other tumors based solely on RCM examination was achieved with a PPV of 94.22%. Malignant tumors (MM and basal cell carcinoma) as a group were diagnosed with a PPV of 96.34%. Classification and regression tree analysis yielded a 3-step algorithm based on only 3 RCM features. This algorithm facilitated a correct classification of 96.30% of MMs, 98.89% of benign nevi, and 100% of basal cell carcinomas and seborrheic keratoses. However, there was a major drawback of both studies: each case was presented to the 2 observers with 2 preselected images leading to a selection bias with increasing probability that observers would evaluate the tumors correctly. Consequently, 3709 unselected melanocytic tumor images derived from randomly selected 20 MMs and 50 benign nevi were evaluated in a retrospective manner, leading to sensitivity and specificity of 97.5% and 99%.[28,29]

Including all RCM images in each melanocytic skin tumor (approximately 163 images per lesion; 16,618 images: 5906 MM, 7693 acquired nevi, 3019 epithelioid and/or spindle cell nevi), Pellacani et al conducted a study to describe melanocytic RCM features and to evaluate their diagnostic significance to MM detection.[12] In a multivariate analysis, 6 criteria were identified as independently correlated with a MM diagnosis (2 major criteria: cytologic atypia, nonedged papillae at basal layer; 4 minor criteria: roundish cells in superficial layers spreading upward in a pagetoid spread, pagetoid cells widespread throughout the tumor, cerebriform clusters in the papillary dermis, nucleated cells within dermal papilla). Using a scoring system, a sensitivity of 83.80% and a specificity of 96.90% were found. As a major pitfall in RCM diagnosis, Spitz nevi presenting "malignant" criteria were described (see above).

More recently, Pellacani et al evaluated a study set comprising 136 MMs and 215 nevi by RCM.[30] Thirty-seven RCM features were evaluated by 2 blinded observers to identify significant features and to test diagnostic models. A diagnostic algorithm as proposed in their previous study resulted in a sensitivity of 91.9% and specificity of 69.3%. Interestingly, identification of pagetoid infiltration was found to represent the most relevant discriminant feature, whereas normal-appearing epidermal architecture seemed to be specific for benign lesions (see above).

Langley et al[31] conducted the first study evaluating the diagnostic accuracy of RCM compared with dermoscopy in a prospective examination of melanocytic skin tumors. Overall, 37 MMs and 88 nevi were studied (30,089 images: 18,045 nevi, 12,044 MM). Examination was performed by a single observer with experience in RCM. Sensitivity and specificity of dermoscopy for the diagnosis of MM were 89.2% and 84.1% (PPV 70.2%, NPV 94.9%), respectively. Sensitivity and specificity of RCM were 97.3% and 83.0% (PPV 70.6%, NPV 98.6%), respectively. RCM had a higher sensitivity with similar specificity compared with dermoscopy; however, using McNemar's test of significance for sensitivity and specificity combined, no significant difference was found between the sensitivities and specificities of the 2 methods.[28]

Future Prospects

RCM is an exciting new tool for in vivo imaging of the skin. The technology holds significant promise for the future. Improvement of diagnostic accuracy, improved assessment of dermoscopic-histologic correlation, in vivo biopsy side selection, surgical margin assessment, and response control of conservative therapies in skin diseases are some of the major advantages of this novel imaging method. Additionally, RCM holds inherent potential for teledermatologic application and automated image analyzing.

Early detection of MM of the skin represents one of the greatest challenges in dermatology. Although surgical excision in early stages of MM development is almost always curative, delayed recognition puts the patient at risk for destructive growth, metastasis, and death from disease. The introduction of dermoscopy has led to improved diagnostic accuracy for melanocytic skin tumors. Dermoscopy has been tested for diagnostic accuracy in melanocytic skin lesions in a large number of studies. Remarkably, in a recent meta-analysis of 13 dermoscopic studies, a sensitivity of 83.2% and specificity of 85.8%, both superior to the diagnostic accuracy achieved with the unaided eye,[32,33] were reached by the method.[34] In contrast, taking into account all RCM studies dealing with diagnostic accuracy in melanocytic skin tumors, sensitivity and specificity of approximately 90% and 86% could be found.[12,24,27,28,30,31] This improvement in sensitivity is in line with results presented by Langley and coworkers, comparing diagnostic accuracy of dermoscopy and RCM in a prospective examination.[31] Whereas dermoscopy has probably reached the method's inherent potential diagnostic accuracy because of the lack of cellular level evaluation, further improvements could be expected by RCM in the oncoming years. For example, in dermoscopy the presence of a blue hue is a clue for malignancy. However, dermoscopic differentiation between a blue whitish veil, which was reported to be specific for MMs, and blue areas, which are frequently found in benign lesions, is not adequately possible, although a dif-

ferent histologic substrate was described. Pellacani et al[16] conducted a study, including 213 melanocytic skin tumors with 84 lesions representing a blue hue to identify the in vivo microscopy correlates of the blue hue. RCM appeared to permit a differentiation of the substrates of the blue areas and blue veil, enabling the visualization of features consistent for malignancy.

Another useful advantage of RCM is that it enables detection of amelanotic MM noninvasively.[10] RCM allows recognition of abnormal intraepidermal melanocytic proliferation in clinically amelanotic MM that is distinct from features of normal skin. These features may be evident because of the presence of melanosomes or melanin-filled premelanosomes in the cytoplasm. However, prospective large-scale studies evaluating RCM features of amelanotic MM are lacking.

To avoid repeated invasive surgical management and consequent scarring and complications, conservative, noninvasive therapies for cutaneous cancers have been developed. However, the efficacy of the applied treatment could only be histologically assessed by invasive skin biopsies. RCM could be a useful adjunct tool for monitoring of treatment response, as has been described, for example, for the effects of cryotherapy in basal cell carcinoma.[35] In vivo biopsy site selection and in vivo surgical margin detection are other examples for the usefulness of RCM in treatment management.

A limitation to the current state of RCM technology that has to be addressed is that imaging is restricted to a depth of 350-500 μm, which corresponds to the papillary dermis and, depending on skin thickness, the superficial reticular dermis. Therefore, assessment of microanatomical structures in the reticular dermis or tumor invasion depth cannot be evaluated reliably. The presence of refractive structures may also decrease contrast and make melanocyte visualization difficult. This might be improved by testing of different immersion media and illumination sources. As in other new imaging technologies, image interpretation is difficult for the untrained observer. However, it has been reported that less time is needed to become proficient in confocal microscopy than for formal training in dermoscopy.[24,36]

The first generation of confocal microscopes had a complex and bulky configuration impeding convenient attachment to certain anatomical areas (Fig. 1). One single imaging session was very time-consuming. As a consequence, great efforts have been made to design compact instruments for everyday use in routine clinical application. Recently, the first handheld confocal microscope was introduced. The new generation of microscopes is small and easy to employ. Furthermore, it offers the possibility of coupling a digital camera, by which a clinical still image can be acquired (Fig. 2A). By navigating through the clinical image field to specific regions of interest which can be assessed with the confocal microscope, a correlation between the macroscopic and the confocal images is provided (Fig. 2A-C). This system provides a synergistic multimodal combination for routine clinical application.

Because RCM images are viewed 2-dimensionally on a computer screen, they are ideally suited for teledermatology application (Fig. 2A-C). RCM images can be transferred to a remote specialist for a second opinion. The captured RCM images can be uploaded to a server via a secure connection. From this server, images can be retrieved by authorized specialists. This store-and-forward method is time-independent and does not require the presence of the involved medical experts at the same time. A real-time observation of RCM images by a remote observer could be possible in the near future.

Furthermore, image analysis systems highlighting diagnostic morphologic image regions and automatic diagnostic image analysis procedures are under development. Key morphologic features have already been described and several studies provide evidence for high diagnostic accuracy in melanocytic skin tumors. The studies described above represent a significant contribution to the body of research necessary for the evaluation and implementation of RCM in clinical practice to avoid many currently unnecessary biopsies. RCM probably augurs a sea of change in the way we evaluate melanocytic skin tumors in the future and will ultimately move the art of histologic diagnosis closer to the bedside.[37,38] The future looks bright for RCM.

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