Introduction to reflectance confocal microscopy and its use in clinical practice

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Reflectance confocal microscopy (RCM) is a novel technology that provides noninvasive, in vivo imaging of the skin at nearly histologic resolution. In 2016, the US Centers for Medicare and Medicaid Services (CMS) established reimbursement codes for RCM image acquisition and for the reading and interpretation of images. The combination of RCM imaging with dermoscopy has improved the accuracy of skin cancer diagnosis while reducing the number of biopsies of benign skin lesions. With that, we are starting to see more dermatologists and dermatopathologists using RCM in clinical practice. This editorial is to serve as an introduction on RCM imaging with a focus on its usefulness in both the diagnosis and management of skin cancers. We end by briefly describing the characteristic RCM features of normal skin to serve as a building block for later cases that will explore both the benefits and drawbacks of incorporating RCM imaging for benign and malignant lesions. (J Am Acad Dermatol 2018;4:1014-23.)

Key words: innovative technology; lentigo maligna; melanoma; noninvasive imaging; nonmelanoma skin cancer; reflectance confocal microscopy; skin cancer.

INTRODUCTION

Reflectance confocal microscopy (RCM) is a US Food and Drug Administration–approved optical imaging technology that offers noninvasive visualization of skin lesions in vivo at nearly histologic resolution. In 2016, the Centers for Medicare and Medicaid Services (CMS) granted category I current procedural terminology (CPT) codes (96931-96936) for RCM imaging and evaluation of skin lesions.[1] Physicians can now submit a procedural bill for potential reimbursement for the cellular and subcellular image acquisition or interpretation and report of skin lesions.[1] Although the cost of purchasing a device has previously limited its use to large academic and research centers, now with reimbursement and the option to lease, we predict that this technology will gain more traction in the United States market. With this comes the need to narrow the educational gap hindering dermatologists from using this device in clinical practice.

Abbreviations used:

CMS:	US Centers for Medicare and Medicaid Services
CPT:	current procedural terminology
LM:	lentigo maligna
NNT:	number needed to treat
RCM:	reflectance confocal microscopy

RCM TECHNICAL PROPERTIES

The current commercially available in vivo devices include the wide-probe RCM, VivaScope 1500 (Caliber Imaging and Diagnostics, Rochester, NY) and the handheld RCM, VivaScope 3000 (Caliber Imaging and Diagnostics). RCM imaging provides nuclear and cellular morphology of the skin with a typical lateral (ie, horizontal) resolution of 0.5 to 1 μm and axial resolution (ie, vertical layer thickness) of between 3 and 5 μm, to a depth of about 150 to 200 μm depending on the anatomical site.[2-6] Imaging is in the horizontal (en face) plane, parallel to the skin surface, similar to the field of view obtained in dermoscopy and Mohs sections. The VivaScope 1500 creates individual optical sections in small 0.5- x 0.5-mm fields of view at 30x magnification comparable to histopathology. To image in depth, RCM can create a stack of images at the same horizontal plane

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at sequential depths from the stratum corneum down to the underlying papillary dermis, termed an *optical biopsy*. At any depth of interest, an automated software can stitch together consecutive optical sections at a single preselected depth/plane into a 2-dimensional block, or mosaic, increasing the field of view to up to 8×8 mm^2.

RCM's ability to acquire mosaics and stacks in real time enables noninvasive evaluation of a large area of tissue in vivo. For comparison, RCMs large field of view makes it possible to assess significantly more of the lesion than what is usually examined in routine histopathologic analyses. Therefore, RCM may assist in isolating an area of interest within larger lesions that may yield the most diagnostic information on histology.

The wide-probe RCM requires adhesion of a 2-cm metal ring flat on the skin, making it challenging to image surfaces that are curved, narrow, or relatively inaccessible. The handheld device (VivaScope 3000) facilitates access to concave or tight anatomic sites such as around the nose, ears, and eyes. The VivaScope 3000 has similar resolution to that of the VivaScope 1500; however, its field of view is limited to 1000×1000 μm^2, without the ability to create mosaic images. Additionally, the VivaScope 3000 cannot currently correlate the scan with its macroscopic picture, making localization difficult. This handheld device has no approved CPT code at this time and therefore is not reimbursable.

LIMITATIONS OF IN VIVO RCM IMAGING

Depth

Although the maximum imaging depth is about 300 μm, the imaging resolution decreases substantially below a depth of 100 to 150 μm, restricting accurate diagnostic interpretation to the epidermis and superficial dermis. This limited penetration depth may be insufficient to detect tumor invasion or deep tumor margins. This makes imaging less suitable for lesions that are nodular or have marked epidermal thickening, ulceration, or hyperkeratosis, as these factors reduce image quality and may further prevent adequate imaging penetration depth.[7]

Accuracy

The diagnostic accuracy of RCM imaging is not 100%; therefore, there is a risk of error when compared with histopathology. For example, bright cells in a pagetoid pattern on RCM imaging can represent either melanocytes, which strongly suggest a melanoma, or intraepidermal Langerhans cells, which are benign immune system cells.[8,9] Additionally pagetoid cells infiltrating the epidermis lack diagnostic specificity, as they can also occur in Spitz nevi, acral nevi, nevi of special sites including breast and genital regions, and irritated or inflamed nevi.[8,10] In one study, the presence of bright cells in a pagetoid pattern on RCM imaging led to a false-positive diagnosis of melanoma in 24 of 39 cases assessed.[8] It is often not possible to distinguish severely dysplastic nevi from malignant melanoma.[11] Alternatively, the presence of inflammatory infiltrates can hide features of an underlying melanoma resulting in a false-negative diagnosis.[2]

Training requirements

It is estimated that a minimum of 4 to 6 months of training, including the evaluation of several thousands of cases, is required for a clinician to reach an acceptable level of diagnostic accuracy and expertise.[12,13] However, with the development of a HIPAA-compliant telemedicine server, Vivanet (Caliber ID), RCM images can be sent through to a remote reader for image interpretation or a second opinion, which allows for separate reimbursement for image acquisition and interpretation, similar to the reimbursements with conventional biopsy and histopathology interpretation.[12,14]

Efficiency

RCM imaging takes significantly longer than dermoscopy and therefore should not be used as a replacement to dermoscopy as a screening tool but as an adjunct in selecting equivocal lesions of concern based on dermoscopic findings. Most of the studies calculating diagnostic accuracy include prior clinical and dermoscopic data; therefore, it is optimal to be proficient in dermoscopy to efficiently and effectively decide which lesions should subsequently be referred for RCM imaging and the actual interpretation of these lesions.

IMAGING PROTOCOL AND REIMBURSEMENT?

The CPT codes provide reimbursement on a per-lesion basis, similar to the reimbursements for routine biopsy and pathology. Actual reimbursement varies by locality and can be found on the CMS website. Currently, only the wide-probe VivaScope 1500 has approved CPT codes. The first 3 codes (96931-96933) are used when imaging a single lesion on a patient.[1] The code 96931 is used when image acquisition, reading, and interpretation is all done by a single clinician.[1] Alternatively, if separate clinicians carry out each task, then 96932 is billed for image acquisition only, and 96933 is for generating a report only.[1] The next 3 codes (96934-96936) are used for

each additional lesion and are designed parallel to the setup of the first 3 codes.[1]

For use of the CPT code, the CMS requires the acquisition of 3 to 5 mosaics at different depths and the optional acquisition of stacks at various foci of concern. To start imaging, you must manually set the zero depth (z = 0) to the topmost surface of the skin (stratum corneum), as this acts as a reference marker for your entire scan. To obtain accurate image analysis for diagnostic purposes, mosaic images must be obtained at the suprabasal epidermis, the dermoepidermal junction, and the papillary dermis. The ability to accurately identify each layer requires extensive training and is therefore operator dependent. Another option is to automate the process by choosing mosaics based on predefined depths; however, this increases the risk of potentially missing a clinically important level.

PRACTICALITY FOR USING CONFOCAL IN A PRACTICE

Diagnosis

RCM imaging has been shown to significantly improve diagnostic accuracy and early detection of melanocytic and nonmelanocytic skin cancers compared with clinical and dermoscopic examination alone.[15-20] A recent meta-analysis that evaluated 21 studies with a total of 3602 lesions found that the pooled sensitivity and specificity was 93.6% (92%-95%) and 82.7% (81%-84%), respectively, for all skin cancers.[16] Particularly, RCM had a sensitivity of 92.7% (90%-95%) and specificity of 78.3% (76%-81%) for detecting melanoma and a sensitivity of 91.7% (87%-95%) and specificity of 91.3% (94%-96%) for detecting basal cell carcinoma.[16] Although the sensitivity for RCM and dermoscopy were similar and insignificant, RCM significantly increased the specificity for the detection of both malignant skin tumors and melanoma compared with dermoscopy alone.[16,19]

Lentigo maligna (LM) and lentigo maligna melanoma, melanoma subtypes on chronically sun-damaged skin, are often diagnostically challenging to both dermatologists and dermatopathologists because of ill-defined borders and overlapping features with solar lentigines, pigmented actinic keratosis, lichen planus–like keratosis, and seborrheic keratosis.[21] These lesions are often large in diameter and/or located near cosmetically sensitive areas making an excisional biopsy impractical and unfeasible. Small incisional biopsies at the darkest and/or thickest areas of the lesion do not always correlate with the most advanced areas histologically, as there are significant histologic variability and skip features within a given lesion; therefore, limited sampling may be inadequate for diagnosis.[22] RCM imaging has been shown to have a sensitivity of 85% and specificity of 76% for LM diagnosis and therefore can help to select specific foci for an incisional or partial biopsy that will best establish the diagnosis and reduce sampling error.[23]

RCM has been especially useful in detecting hypomelanotic and amelanotic melanomas, achieving a sensitivity of 85% and specificity of 84%.[15] In this study, the specificity for RCM was significantly higher than that for dermoscopy (39%; $P < .001$) as well as for the specificity for pigmented melanomas (65%; $P < .001$).[15] These dermoscopic featureless or structureless lesions are diagnostically challenging; however, RCM enables the visualization of highly refractile melanocytes, including melanocytes in amelanotic tumors, even though these lesions lack pigment on dermoscopy.[15,24-27]

When used as a second-level examination on dermoscopically equivocal lesions, RCM imaging can improve our ability to differentiate benign from malignant skin lesions, significantly reducing the number of unnecessary biopsies by 50% to 70%, thereby decreasing its associated morbidity and health care expenditures.[15,17,18,28-30] The use of dermoscopy and RCM imaging together can decrease the number needed to treat (NNT) ratio by about 2 times relative to that with dermoscopy alone.[17,18,28,30] One study found the NNT a melanoma decreased from 14.6 to 6.8 using RCM.[28] Similarly, another report found a NNT of 19.41 when only dermoscopy was used, which decreased to 6.25 when dermoscopy and RCM imaging was combined.[30] This finding is especially significant when assessing for neoplasms in cosmetically and anatomically sensitive areas such as the face, head, and neck, in which biopsies can result in disfiguring scars, and for sites with increased risk of hypertrophic scarring (eg, upper chest) or infection and delayed wound healing (eg, lower extremities). In addition, noninvasive imaging can greatly improve quality of life in patients with many atypical nevi, such as in patients with atypical mole syndrome or genodermatoses who are at risk for multiple skin cancers, as these patients are subjected to multiple repeated biopsies.[24,31-33] The use of RCM in high-risk patients with equivocal melanocytic flat lesions on dermoscopy that display change on digital follow-up has been shown to reduce the number of excisions of benign lesions while also improving the ability to detect subtle early melanomas.[32,33] However, as with histopathology, dysplastic nevi and melanoma have overlapping features on RCM imaging; therefore, the decision not to excise dermoscopic changing lesions

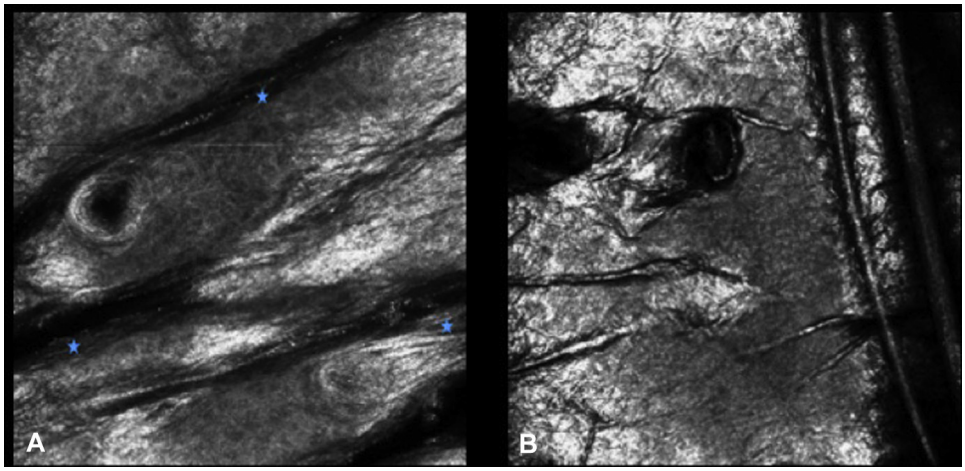

Fig 1. **A**, RCM image (0.5 × 0.5 mm) of stratum corneum in normal skin. Dark linear furrows (*blue star*) represent dermatoglyphs (skin folds). **B**, Close up of anucleated corneocytes in the stratum corneum.
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that appear benign or dysplastic on RCM should continue to be evaluated in further studies.[11]

RCM imaging is also useful in the diagnosis of inflammatory and infectious skin disorders, including eczema, psoriasis, fungal hyphae, scabies, and *Demodex* mites.[34-36] Additionally, RCM can be used to evaluate mucosal lesions such as those located in oral or genital regions.[37,38]

Treatment/management of skin cancers

In addition to diagnosis, RCM has a potential role in guiding the surgical and nonsurgical management of skin cancers. RCM has been found to accurately delineate lateral tumor margins of both melanoma and nonmelanoma skin cancer before surgery, potentially reducing the number of surgical layers.[39,40] This can be especially useful for lesions with ill-defined borders, particularly on sun-damaged skin on the head and neck area, and for lesions that are hypomelanotic or showing regression structures on dermoscopy.[9,15,17,25,26,41-43]

In one study, 17 of 29 (59%) LM lesions were found to have evidence of subclinical disease on both RCM and histopathology that extended more than 5 mm beyond the edge of the dermoscopically identified margin.[39] Both the length and width of the clinically visible LM were on average 60% smaller than the final corresponding dimensions determined by RCM assessment.[39] RCM mapping of these difficult/challenging LM and lentigo maligna melanoma changed the management in 27 of 37 patients (73%), with 11 having a major revision in surgical excision and the remaining given topical imiquimod or radiotherapy.[39]

For lesions that are obviously malignant on RCM imaging, the clinician may decide to do a definitive surgical excision rather than a small partial biopsy, thereby achieving the result in one step. We suspect that in the future, RCM imaging will enable both the diagnosis and treatment to take place on the initial consultation day, obviating the need for the initial diagnostic biopsy.[44] One study showed that this one-stop-shop concept was as efficacious/noninferior in achieving tumor-free margins and significantly increased patient satisfaction in terms of convenience compared with standard of care.[44]

Nonsurgical treatment monitoring

As nonsurgical therapies continue to emerge as either an alternative or an adjunct to surgery, RCM imaging can be used to monitor treatment efficacy. Often patients refuse a biopsy to confirm clearance; therefore, clearance rates are determined clinically. However, local side effects, such as erythema or hyperpigmentation, may obscure residual tumor or be misinterpreted as cancer persistence, resulting in additional unnecessary treatment. By leaving the underlying skin intact, RCM allows for longitudinal surveillance to evaluate for the persistence or recurrence of lesions, eliminating the need for a posttreatment biopsy. Few studies have found the feasibility of RCM imaging in evaluating the efficacy of these less-invasive therapies, including image-guided laser ablation, radiation therapy, topical imiquimod therapy, photodynamic therapy, and oral hedgehog inhibitors.[45-54] However, because of limited imaging penetration depth, caution must be taken to miss deep residual disease.

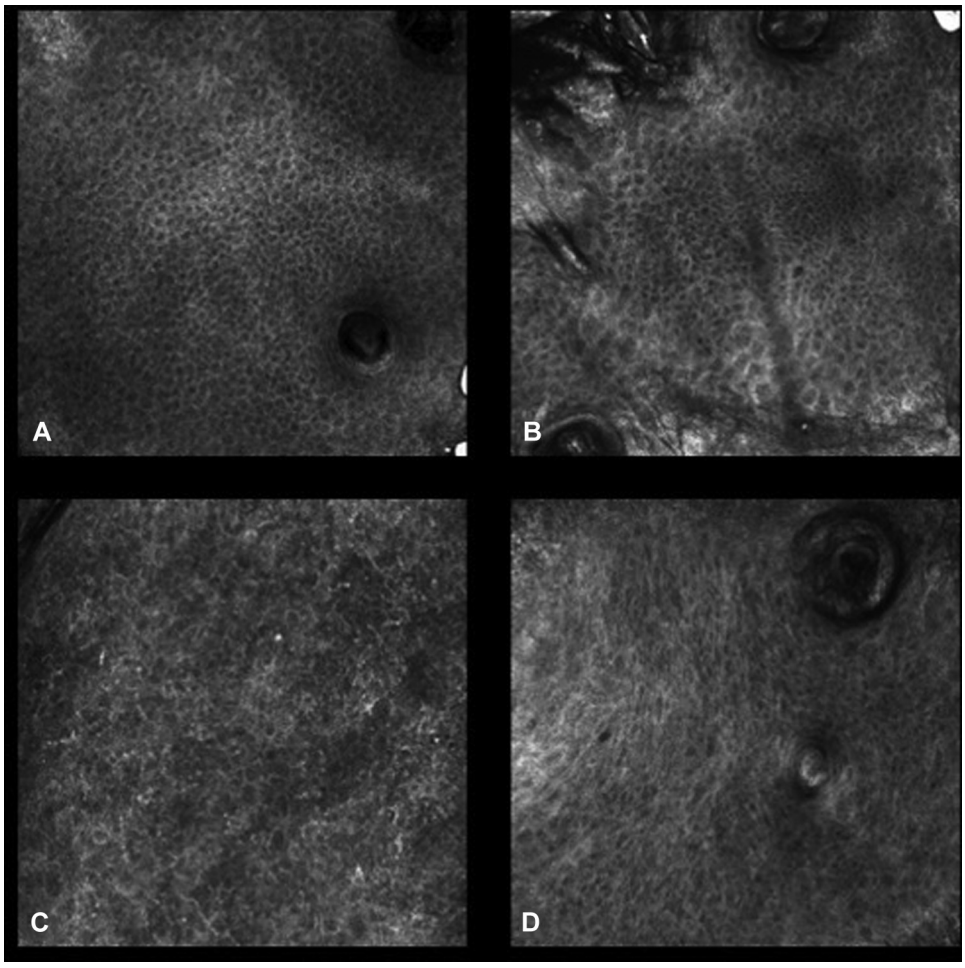

Fig 2. A, RCM image (0.5 × 0.5 mm) of normal honeycomb pattern at the stratum granulosum—spinosum level. **B**, RCM image (0.5 × 0.5 mm) of a broadened honeycomb pattern at the stratum granulosum—spinosum level. **C** and **D**, RCM image (0.5 × 0.5 mm) of an irregular honeycomb pattern as displayed by a disruption of the honeycomb pattern with cells of variable size, shape and contour thickness.
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NORMAL SKIN FEATURES ON RCM

To recognize pathologic features from abnormal skin, it is first essential to know and recognize the characteristic findings of normal skin and benign lesions at each anatomic level on RCM. To be reimbursed for image acquisition, CMS mandates mosaic images at the following levels: stratum corneum, stratum granulosum, stratum spinosum, the dermoepidermal junction (DEJ), and the papillary dermis. Visualization of the DEJ is crucial, as most skin cancers originate and spread from the basal layer, and as such this is often the first mosaic that dermatologists and dermatopathologists will review.[2] Of note, given that most anatomic regions have convexities and concavities, it is likely that you will see multiple layers in one mosaic; however, it is important to obtain multiple mosaics for accurate diagnosis as well as reimbursement purposes.

RCM uses a near-infrared laser (830-nm wavelength) to produce high-resolution images based on differences in the reflection and backscattering of light from the examined tissue section.[2,55,56] Highly refractive/reflective structures, including melanin, keratin, and collagen, appear bright/white providing contrast to surrounding tissue.[57-60] Melanin produces the strongest contrast, allowing for the recognition of melanocytes, melanophages, and pigmented keratinocytes.[57-61] As such, the appearance of normal skin

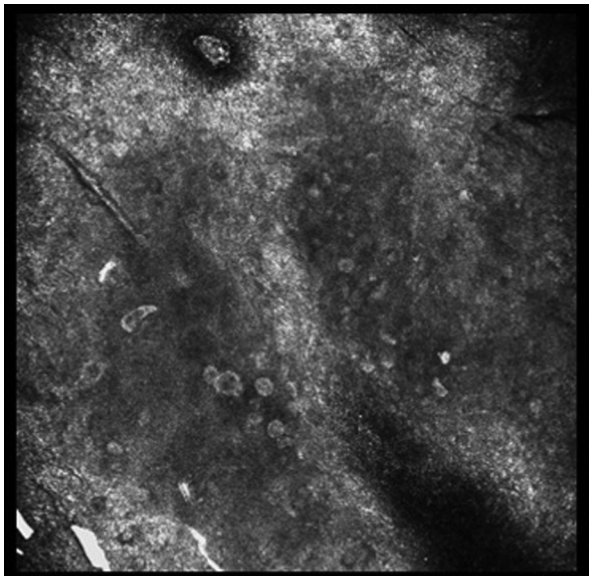

Fig 3. Disarranged pattern in a biopsy-proven melanoma displays a disarray of normal architecture of the superficial layers with unevenly distributed bright granular particles and cells, in the absence of honeycombed or cobblestone. This pattern is more frequently observed in invasive melanomas, usually associated with pagetoid cells although can be seen in other skin malignancies.
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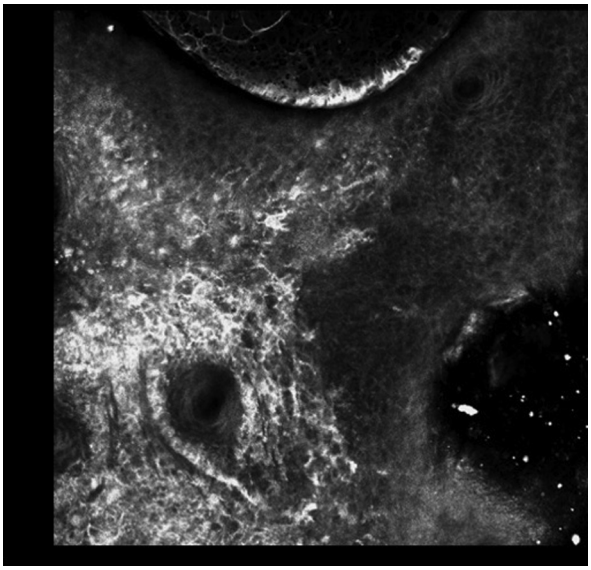

Fig 4. RCM image (0.5 × 0.5 mm) of bright cells in pagetoid spread and dendritic cells extending down the follicular infundibulum in a biopsy-proven lentigo maligna.
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varies among different skin phototypes and anatomic locations.[61] In patients with darker skin color (phototype II-IV) or in melanocytic/pigmented lesions, the pigmented basal keratinocytes and melanocytes are easily identifiable as melanin is a natural source of contrast in RCM images.[62-64] In lighter skin phototypes (I-II) the basal keratinocytes have low refractility and dermal papillary rings/DEJ are difficult to elucidate.[61,63-65]

Stratum corneum

RCM imaging typically begins at the skin surface and progresses downward to the papillary dermis. The first layer encountered is the stratum corneum, located 0 to 20 μm from the skin surface. This layer is composed of large polygonal-shaped, anucleated corneocytes and appears as a bright, highly reflective surface separated by dark furrows representing skin folds (dermatoglyphs) (Fig 1).[62-64] The total thickness of the corneal layer and the depth of the skin folds varies due to anatomic location and degree of sun exposure.[62]

Stratum granulosum and stratum spinosum

The stratum granulosum (or granular layer), located 15 to 25 μm below the skin surface, and its underlying stratum spinosum, which extends from about 25 to 100 μm in depth, consists of large polygonal cells with dark nuclei surrounded by bright grainy cytoplasm resembling a regular honeycombed pattern (Fig 2).[62-64,66] Spinous keratinocytes are smaller than granular keratinocytes with a progressive reduction in cell diameter with depth.

An irregular (atypical) honeycomb pattern displays a disruption of the characteristic honeycomb pattern with cells of variable size, shape and contour thickness (Fig 2, *C* and *D*).[6,63,66,67] This is typically found in malignancy including actinic keratosis and squamous cell carcinoma.[66]

A broadened honeycomb pattern shows polygonal outlines that are uniformly thickened and brighter, correlating to an acanthotic epidermis (Fig 2, *B*).[6,63] This is seen in some seborrheic keratosis, solar keratosis, nodular melanomas, epithelial tumors, and some Spitz nevi.[64]

Finally, a disarranged epidermal pattern is characterized by the absence of honeycombed or cobblestone pattern and is usually associated with malignancy (Fig 3).[6,63,64,67]

Langerhans cells can also be found in the spinous epidermal layer, and when activated they appear as highly refractile dendritic cells, often exhibiting a stellate morphology, and must be differentiated from dendritic melanocytes (Fig 4).[8,63,64,68]

Stratum basalis – dermal epidermal junction

The stratum basalis (or basal layer), is located at a depth of about 50 to 100 μm from the skin surface and is a single layer of basal cells situated just above

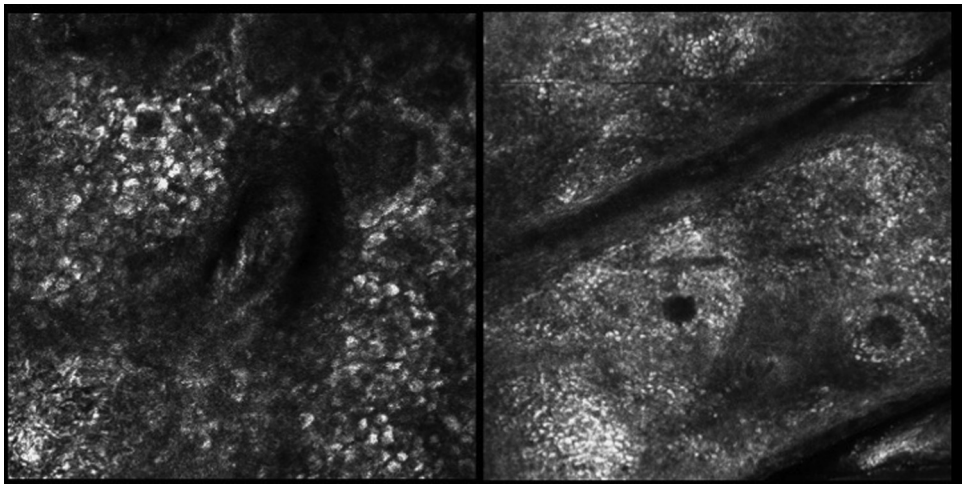

Fig 5. Pigmented basal keratinocytes at the suprabasilar layer displaying a cobblestone pattern owing to the high refractive index of their supranuclear melanin caps.
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the basement membrane at the DEJ. Basal cells are typically the smallest keratinocytes visible in the epidermis and appear as bright clusters of round cells owing to the high refractive index of their supranuclear melanin caps, forming a cobblestone pattern at the top of the basal layer (at the suprabasal layer or suprapapillary plates) (Fig 5).[62-64,66] Melanocytes are bright round to oval or elongated fusiform cells that are best recognized by the presence of bright dendritic processes.[27] However, when these dendrites are not identified, they can be difficult to differentiate from keratinocytes or Langerhans cells.[8,68] In these cases, to best distinguish melanocytes from other cells it is necessary to use architectural content in addition to cellular morphologic features.[68] Melanocytes appear bright in signal intensity diffusely throughout the cytoplasm, and they are seen as solitary units or nests positioned at the DEJ.[68]

As you descend deeper into the DEJ (50-150 μm), the dermis forms upward fingerlike projections into the epidermis called *dermal papillae*. Because of the en face orientation of RCM images, dermal papillae appear as dark round to oval areas surrounded by a rim of bright basal keratinocytes and melanocytes, termed *dermal papillary rings* (edged papillae) (Fig 6).[62,67] These rims increase in size until they reach the rim of the surrounding papillae, which signifies that the base of the dermal papillae has been reached. In areas with flattened rete ridges, this pattern is less discernable.[63,64] Blood flow in capillary loops can be observed within the dermal papillae.

When papillary contours are not well outlined, they are referred to as *nonedged papillae*, which is concerning for melanoma, although it can be found in Spitz nevi, atypical nevi, and as compound/congenital nevi.[23,63,64,67]

Papillary dermis

Below the DEJ lies the papillary dermis (100-150 μm) and superficial reticular dermis (>150 μm) which appears less refractile and contains dark tubular or canalicular blood vessels and bright collagen fibers (Fig 7).[64] The collagen fibers appear in a reticulated or web-like pattern in the papillary dermis and as large fascicle bundles in the reticular dermis.[64] In chronically photo-exposed skin sites, there is an increase in volume and irregularity of the collagen bundles indicative of solar elastosis.[64] Dermal inflammatory cells, including melanophages and leukocytes, can also be visualized in the perivascular area.[64] Melanophages are usually larger in size than melanocytes and appear as irregularly shaped, plump, variably bright cells with fuzzy cell borders located around papillary dermal capillaries.[68] At times, different collagen patterns and atypical cells that migrate down from other layers may be present to enhance a diagnosis, but because of reduced resolution and limited imaging depth, findings in the dermis are mainly used to confirm diagnosis.

CONCLUSION

With the implementation of new diagnostic CPT codes for RCM imaging, physicians can now be

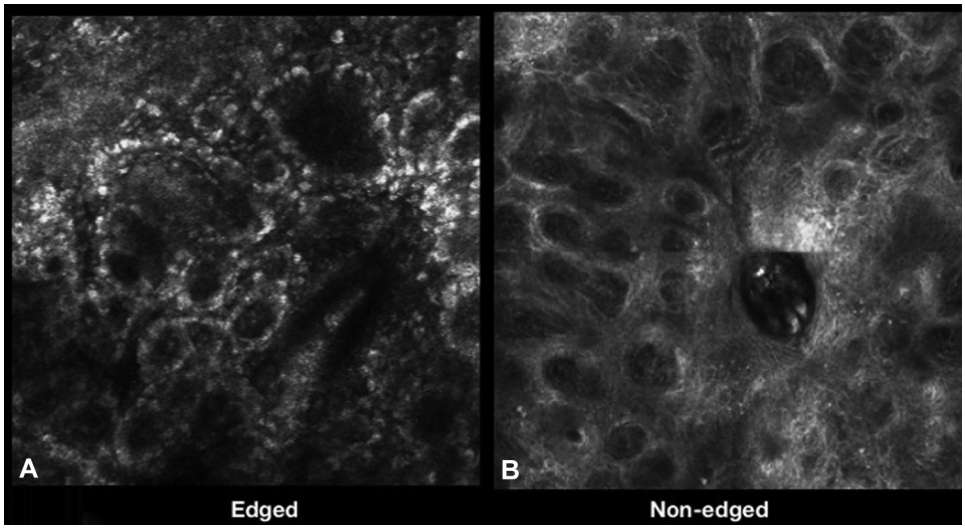

Fig 6. A, RCM image (0.5 × 0.5 mm) of dermal papillae surrounded by bright basal keratinocytes and melanocytes (edged papillae) at the DEJ. **B**, RCM mosaic (1.0 × 1.0 mm) of papillary contours that are not well outlined (nonedged) in a melanoma in situ.
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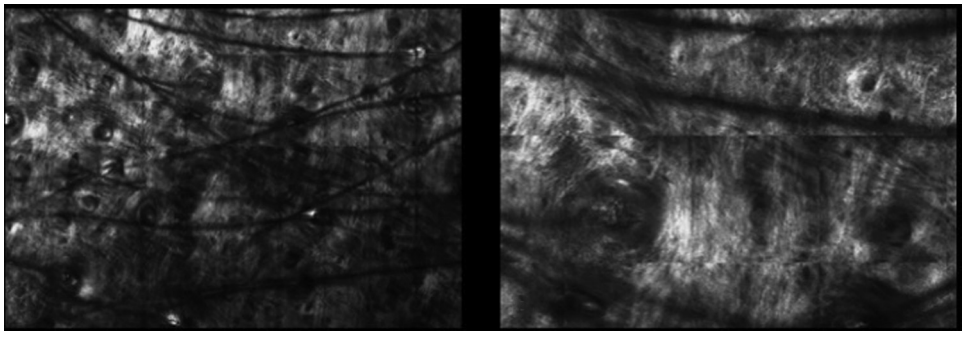

Fig 7. RCM mosaic of the superficial dermis, which appears less refractile and contains dark tubular or canalicular blood vessels and bright collagen fibers.
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reimbursed for the acquisition and interpretation of skin lesions. RCM may improve the diagnostic accuracy and early detection of equivocal skin lesions and reduce the number of unnecessary excisions of benign tumors, thereby decreasing its associated morbidity and health care expenditures. In a clinical setting, RCM imaging will benefit patients by providing same-day diagnosis of a cutaneous disease as well as confirmation of treatment efficacy, or as a perioperative tool to aid in cutaneous surgeries. RCM's ability to scan the entire lesion and non-invasively determine the most diagnostically and prognostically significant area to biopsy will help to reduce the risk of sampling error and false-negative rates owing to heterogeneity within lesions. RCM imaging is a novel technology in dermatology that will continue to evolve and become more widespread in upcoming years.

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