STUDY

Reflectance Confocal Microscopy Criteria for Squamous Cell Carcinomas and Actinic Keratoses
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Objective: To identify criteria for the diagnosis of squamous cell carcinoma (SCC) and actinic keratosis (AK) by in vivo reflectance confocal microscopy (RCM).

Design: Prospective RCM imaging of lesions suspected clinically and/or dermoscopically to be SCC or AK, followed by RCM assessment of the biopsy-proven SCCs and AKs.

Setting: Private skin cancer clinic, Plantation, Florida.

Patients: A total of 38 lesions in 24 patients were assessed, including 7 AKs, 25 SCCs in situ, 3 invasive SCCs, and 3 keratoacanthomas.

Interventions: Prior to undergoing biopsy, all lesions were assessed by RCM.

Results: Mosaic RCM images at the stratum corneum level revealed scale in 29 SCCs (95%) and in all 7 AKs. Polygonal nucleated cells at the stratum corneum were seen in 3 SCCs (10%) and 1 AK (14%). All 38 cases displayed an atypical honeycomb or a disarranged pattern of the spinous-granular layer of the epidermis; round nucleated cells were seen in the spinous-granular layer in 20 SCCs (65%) and 1 AK (14%). Round blood vessels in the superficial dermis were seen in 28 SCCs (90%) and 5 AKs (72%).

Conclusions: An increasing frequency of abnormal RCM features can be observed across the spectrum of keratinocytic neoplasias. The presence of an atypical honeycomb or a disarranged pattern of the spinous-granular layer, round nucleated cells at the spinous-granular layer, and round blood vessels traversing through the dermal papilla are the key RCM features of SCC.

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QUAMOUS CELL CARCINOMA (SCC) is one of the most common cutaneous neoplasms. The classic presentation is a scaly, red plaque with or without ulceration. However, SCC may be occasionally difficult to differentiate by clinical appearance from other disease entities. The early recognition of SCC is important because if allowed to proliferate unabated, the neoplasm may acquire the ability to metastasize. Actinic keratoses (AKs) are considered by some as precancerous lesions, while others consider them an incipient form of SCC. Histologically, SCC is a malignant neoplasm of epidermal keratinocytes that demonstrate crowded, enlarged, and pleomorphic nuclei; abundant eosinophilic cytoplasm; and signs of abnormal cornification that may include dyskeratosis and parakeratosis. Histologically, AK tends to show a milder degree of keratinocytic atypia that is confined to the lower part of the epidermis. Reflectance confocal microscopy is a relatively new noninvasive imaging technique that has shown promise as a diagnostic aid in many dermatologic conditions. It helps to bridge the gap between dermoscopy and histologic analysis, allowing horizontal evaluation of a lesion (as
with dermoscopy) while producing in vivo images of the epidermis and superficial dermis at a resolution that approximates that of histopathologic specimens.4,5 To our knowledge, few publications describe the RCM features of SCC and AK based on small case series.6–10

The aim of the current study was to propose a set of well-defined, reproducible RCM diagnostic criteria for SCC. We evaluated RCM images from a prospectively imaged series of histologically confirmed keratinocytic neoplasms, ranging from AK to SCC, using previously and newly described RCM attributes. This is a necessary step toward the goal of using RCM to discriminate SCC from other disease entities that present as red scaly plaques.

### METHODS

Due to the nature of the study, the institutional review board and human study committee approvals were waived.

### PATIENTS

We prospectively imaged with RCM skin lesions that were clinically and dermoscopically suspected to be SCCs and AKs. Prior to the biopsy, all lesions were photographed clinically with a Nikon D70 camera (Nikon, Melville, New York) and dermoscopically with the 3 Gen-Dermlite II HR (3Gen, San Juan Capistrano, California) attached to a Sony Cyber-shot camera (Sony, Tokyo, Japan). For this study, we analyzed only lesions that proved histopathologically to be AKs, SCCs (in situ or invasive), or keratoacanthomas.

### RCM IMAGING

To image the lesions by RCM, a drop of high-refractive index cosmetic oil (Crodamol STS; Croda Inc, Edison, New Jersey) was placed on the lesion. A disposable, low birefringence polycarbonate window was adhered to a metal tissue ring, which was then secured to the skin over the oil drop with a medical-grade adhesive (3M Inc, St Paul, Minnesota). Macroscopic images, which allowed for viewing dermoscopic structures within the RCM software interface, were captured with a digital camera (VivaCam; Lucid, Inc, Rochester, New York) through the tissue ring. The universal serial bus–powered camera provided a 5.0-megapixel, 10 × 10-mm image with 10-μm resolution at the tissue. This macroscopic image was then cropped to a 1944 × 1944-pixel image to provide a square field view, which could be minimized and viewed simultaneously with the RCM real-time images. When captured through the tissue ring, the macroscopic image correlated precisely to the RCM mosaic images and thus served as a gross map to guide RCM imaging of subregions of the lesion with a high degree of accuracy.

Reflectance confocal microscopy images were captured through the tissue ring with a near-infrared, commercially available device (VivaScope 1500; Lucid Inc), which used an 830-nm laser and a maximum power output of 22 mW. Instrumentation and acquisition procedures have been described previously.4,5 Individual 500 × 500-μm RCM images with 1.0-megapixel resolution were sequentially collected in the horizontal plane and tiled together to create a mosaic image (up to 8 × 8 mm) using automated software. The time to obtain a mosaic image ranged from 4 seconds (1 × 1 mm) to 2 minutes 34 seconds (8 × 8 mm). In all lesions, mosaic images were obtained at the levels of the stratum corneum, spinous-granular layer, and either the dermoepidermal junction or superficial dermis. Live audio-video interleave (AVI) files, or movies, at 9 frames per second were recorded at the superficial dermis or dermoepidermal junction level. The AVI files contain an overlay indicating the motor positions and laser power settings used during video capture.

### EVALUATION OF IMAGES

Lesions were jointly evaluated for the presence of dermoscopic and RCM features by 3 observers (A.R., M.C.O., and H.S.R.). The images were evaluated first globally using the mosaic images and thus served as a gross map to guide RCM imaging of subregions of the lesion with a high degree of accuracy. Reflectance confocal microscopic features used in this study have been described previously,11 are summarized in Table 1, illustrated in Figures 1, 2, 3, 4, and 5, and are viewable in motion in a short video available at http://www.archdermatol.com. Since RCM images were obtained from superficial to...
deep layers of the skin, the features are described in this sequence. Dermoscopic features used in this study were previously described.3

RESULTS

Thirty-four patients were included in the study (79% men [n = 27]; mean age, 69 years; age range, 30-91 years), contributing a total of 38 biopsy-proven lesions including 25 SCCs in situ, 3 invasive SCCs, 3 keratoacanthomas, and 7 AKs. The anatomic distribution of the lesions was from the head and neck area in 26% (n = 10), torso in 5% (n = 2), upper extremities in 32% (n = 12), and lower extremities in 37% (n = 14).

The dermoscopic and RCM features found in the present study are summarized in Table 2. Of 37 lesions evaluated dermoscopically, 33 (89%) displayed an adherent scale. All 7 AKs and 26 SCCs (87%) (including in situ and invasive SCCs and keratoacanthomas) were characterized by an adherent scale on dermoscopy. One AK (14%) and 18 SCCs (60%) showed either dotted or glomerular vessels.

Mosaic RCM images at the stratum corneum level revealed scale in 36 lesions (95%). All 7 AKs and 29 SCCs (94%) revealed scale. Four of these SCCs (12%) did not show scale on dermoscopy. Polygonal nucleated cells at the stratum corneum were seen in 4 lesions (10%), including 1 AK (14%) and 3 SCCs (10%).

All 38 cases displayed an atypical honeycomb and/or a disarranged pattern of the spinous-granular layer. Among the AKs, the atypia was milder and the disarrangement was more focal than in SCCs (Figure 6). Round nucleated cells were seen in the spinous-granular layer in 21 lesions (55%). Of these, 20 (65%) were SCCs, and only 1 was an AK (14%).

Of the 36 lesions examined live for vessels, round vessels in the superficial dermis were seen in 33 cases (92%), including 5 AKs (72%) and 28 SCCs (95%). Of note, these vessels corresponded to the dotted and glomerular vessels seen on dermoscopy in 19 cases. For example, lesion 11 showed glomerular vessels dermoscopically and round vessels on RCM. In addition, 39% of the lesions (4 AKs and 11 SCCs) showed round blood vessels in the superficial dermis on RCM, while dotted or glomerular vessels were not seen on dermoscopy in these lesions. For example, lesion 14 did not show vessels on dermoscopy but demonstrated round vessels in the superficial dermis on RCM at a depth of 255 µm. The presence of a thick scale on the surface of this lesion may explain the difficulty in visualizing vessels with dermoscopy.

Figure 1. Scale. A, In a submosaic view (1000 x 1500 µm), scale is observed as variably refractile, amorphous material at the stratum corneum. B, Corresponding histologic image shows thickened, parakeratotic stratum corneum (hematoxylin-eosin, original magnification x20).

Figure 2. Polygonal nucleated cells at the stratum corneum. A, Polygonal nucleated cells (arrows) at the stratum corneum appear on reflectance confocal microscopy (500 x 500 µm) as thin bright cellular outlines surrounding dark nuclei; these correspond histologically to parakeratotic cells. B, Histopathologic image from the same case shows parakeratosis (hematoxylin-eosin, original magnification x20).
The series included 3 lesions that proved to be invasive SCCs on histopathologic analysis. Of note, 1 invasive SCC showed pleomorphic nucleated cells on RCM at the level of the superficial dermis (Figure 7).

**COMMENT**

Reflectance confocal microscopy is a useful adjunct to clinical and dermoscopic evaluation of cutaneous neoplasms. Features of RCM can be well correlated with dermoscopy in that both imaging techniques offer an en face view of the lesion. In the present study, we demonstrated that although most SCCs display only limited dermoscopic features, namely scale and dotted or glomerular vessels, a wider range of diagnostic descriptors can be observed with RCM.

The presence of scale was observed in 92% of the dermoscopic images and 95% of RCM mosaic images. By itself, the presence of scale may be of limited diagnostic value because it can be present in many benign lesions such as psoriasis and eczema. However, we decided to include scale in the SCC criteria because within the constellation of other RCM features of SCC, it may prove helpful for diagnosis. Scale is histologically represented by orthokeratosis or parakeratosis. We only observed polygonal nucleated cells in the stratum corneum, the RCM correlate of parakeratotic cells, in a minority of the lesions. This probably represents a limitation of our visual interpretation owing to the image resolution of the RCM device.

Atypical honeycomb and/or a disarranged epidermal pattern were observed in all the AKs and SCCs. The presence of these patterns is best seen on high magnification (ie, on the individual RCM images). To the best of our knowledge, these patterns have no clinical or dermoscopic correlates and are unique to RCM assessment of SCC. While the SCCs showed extensive atypia and/or disarrangement of the spinous-granular layer, most of the AKs revealed a focally disarranged or a mildly atypical honeycomb pattern.

On RCM, the round bright nucleated cells were observed at the spinous-granular layer in 55% of the lesions. These cells represent the atypical and dyskeratotic keratinocytes seen in SCCs and AKs on histopathologic analysis. The lower frequency of this RCM feature in AKs compared with SCCs (14% vs 65%) is in line with previous histopathologic observations.

On dermoscopy, dotted and glomerular vessels were seen in 51% of the lesions (n = 18). In the cases that did
not show these vessels on dermoscopy, RCM was particularly useful in making the diagnosis of SCC. Interestingly, 39% of the lesions (n=7) demonstrated round blood vessels in the superficial dermis on RCM, although their dermoscopic correlate, dotted or glomerular vessels, were not seen. We hypothesize that the infrared laser light of RCM penetrates deeper than the visible light of the dermoscope. Despite some compression effect from the RCM tissue ring, patent vessels can be detected without difficulty because RCM imaging is performed at the microscopic level. In contrast, AKs demonstrated sparse round vessels in the superficial dermis. As AKs are smaller and less developed neoplasms, they likely require less extensive blood supply than SCCs.

Three lesions proved to be keratoacanthomas. These tumors showed similar RCM features to the other lesions in this study. This observation is in line with the notion that keratoacanthomas are variants of SCC. In 1 case of invasive SCC, we were able to see atypical nucleated cells in the superficial dermis. Owing to the paucity of lesions that proved to be invasive SCCs in our series, we did not consider this RCM feature in the diagnostic criteria. However, further research may determine whether this feature will allow differentiation between invasive and in situ SCC.

To our knowledge, few RCM cases of SCC have been previously described. Astner et al described a superficial disruption of the stratum corneum, pleomorphic parakeratosis, severe atypical pleomorphism of the epidermis, severe architectural disarray of the epidermis, and atypical aggregates of keratinocytes in the dermis. Some of these features, including the changes at the stratum corneum and spinous-granular layers, are equivalent to our findings. However, we elected not to address the dermal changes in our study owing to the paucity of invasive SCCs included in our series.

The blood vessels traversing through the dermal papilla are a unique finding in our study. A recent study examining the characteristics of AKs and disseminated superficial actinic porokeratoses on RCM found that AKs had architectural disarray, nuclear pleomorphism, and atypical keratinocytes on RCM. These findings are equivalent to the changes at the spinous-granular layer (atypical honeycomb or a disarranged epidermal pattern and round nucleated cells at the spinous-granular layer) observed by our group. In our study, AKs demonstrated focal atypia of the epidermis on RCM and few vessels in the superficial dermis, findings that are similar to, albeit milder than, those of SCC. These findings reflect the con-
cept that AK, in situ SCC, and invasive SCC are a continuous process on the spectrum of keratinocytic neoplasm. They are also in line with the concept of “field cancerization,” which suggests that subclinical preneoplastic cells are present frequently in skin sites surrounding AKs and SCCs. We acknowledge that we may not be able to unequivocally differentiate AK from SCC. Larger studies would be needed to address the issue of whether RCM can help the clinician decide whether a lesion is AK or SCC. In fact, the ability to make this distinction on histopathologic analysis as opposed to considering AK to be in situ SCC has been previously debated.

Reflectance confocal microscopy may be considered time-consuming. Since the majority of skin lesions are easily diagnosed by clinical and dermoscopic evaluation, what role does RCM have in patient care? In our study, RCM imaging took less than 10 minutes per lesion and greatly increased our diagnostic confidence. Increased diagnostic confidence will limit medical er-

Table 2. Dermoscopic and Reflectance Confocal Microscopic Features Found in the Study

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<tr>
<th>Lesion Type</th>
<th>Dermoscopic Features</th>
<th>RCM Features</th>
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<tr>
<td></td>
<td>Scale</td>
<td>Scale</td>
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<tr>
<td>AK</td>
<td>7/7</td>
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</tr>
<tr>
<td>Invasive SCC</td>
<td>2/2^c</td>
<td>3/3</td>
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<td>KA</td>
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Abbreviations: AK, actinic keratosis; IS, in situ; KA, keratoacanthoma; RCM, reflectance confocal microscopic; SCC, squamous cell carcinoma.

a All data are reported as number of features present/number of lesions examined.
b In 2 IS SCCs, vessels were not examined during live confocal imaging.
c Of the 3 invasive SCCs, 1 was not examined dermoscopically.

Figure 6. Actinic keratosis. A, On reflectance confocal microscopy, actinic keratosis shows focal mild atypia at the spinous-granular layer (500 × 500 µm). B, Histopathologic image from the same case shows keratinocyte atypia in the lower half of the epidermis (hematoxylin-eosin, original magnification ×20).

Figure 7. Invasive squamous cell carcinoma. A, This lesion proved histopathologically to be a superficial invasive squamous cell carcinoma showing atypical keratinocytes with pleomorphic nuclei in the superficial dermis (hematoxylin-eosin, original magnification ×20). B, Corresponding reflectance confocal microscopic view (500 × 500 µm) at the level of the superficial dermis shows multiple cells with refractile cellular outline and a central dark nucleus (arrows); the cells are pleomorphic in size.
rors.21 Although the role of RCM in clinical practice has yet to be defined, we believe that RCM will become an invaluable diagnostic tool within the not-too-distant future. In the meantime, it is our responsibility to determine the utility and limitations of RCM, and we hope that the observations made in this study will contribute to this endeavor.

Our study has several limitations including a small sample size, inclusion of only biopsy-proven lesions, and a lack of a control group for comparison and confirmation of the diagnostic features.

In conclusion, our study has showed several reproducible RCM features of SCC, including the presence of scale, polygonal nucleated cells at the stratum corneum, atypical honeycomb or a disarranged epidermal pattern, round nucleated cells at the spinous-granular layer, and round blood vessels traversing through the dermal papilla. Actinic keratoses manifest similar features to a lesser extent. These findings are an important step toward the use of RCM in the diagnosis of SCC at the bedside. Larger series that include additional diagnostic entities, such as basal cell carcinoma, inflamed seborrheic keratosis, and inflammatory lesions are needed to validate the diagnostic utility of RCM for SCC.

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Author Contributions: Dr Rishpon had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Rishpon, Braun, and Rabinovitz. Acquisition of data: Rishpon, Porges, Oliviero, Fox, and Rabinovitz. Analysis and interpretation of data: Rishpon, Kim, Scope, Braun, Marghoob, and Rabinovitz. Drafting of the manuscript: Rishpon, Kim, Scope, Porges, and Fox. Critical revision of the manuscript for important intellectual content: Oliviero, Braun, Marghoob, and Rabinovitz. Administrative, technical, and material support: Rishpon and Fox. Study supervision: Rabinovitz. Expertise in RCM: Marghoob.

Financial Disclosure: Dr Rabinovitz is a clinical researcher for Lucid, the manufacturer of the confocal microscope. Ms Fox is an employee of Lucid and owns stock in the company.


REFERENCES