Reflectance confocal microscopy made easy: The 4 must-know key features for the diagnosis of melanoma and nonmelanoma skin cancers

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Background: Reflectance confocal microscopy (RCM)-based skin cancer diagnosis requires proficiency.

Objective: To identify a short list of key RCM features of skin cancers and test their diagnostic utility.

Methods: We identified key RCM features through consensus among 6 experts using a modified Delphi method. To test the diagnostic utility of these RCM key features, 10 novice RCM readers evaluated a subset of 100 RCM cases from a retrospective data set of benign and malignant skin neoplasms.

Results: From 56 features reported in the literature, the experts identified 18 RCM features as highly valuable for skin cancer diagnosis. On the basis of consensus definitions, these RCM features were further clustered into 2 melanoma-specific key features (atypical cells and dermoepidermal junction disarray), 1 basal cell carcinoma—specific key feature (basaloid cords/islands), and 1 squamous cell carcinoma—specific key feature (keratinocyte disarray). The novice reading study showed that the presence of at least 1 of the 4 key features was associated with an overall sensitivity for skin cancer diagnosis of 91%, with a sensitivity for melanoma of 93%, a sensitivity for basal cell carcinoma of 92%, and a sensitivity for squamous cell carcinoma of 67%, and an overall specificity of 57%.

Limitations: The consensus was based on only six RCM experts and the validation study was retrospective.

Conclusions: A consensus terminology short list identifying the 4 RCM key features for skin cancer diagnosis may facilitate dissemination of RCM to novice users. (J Am Acad Dermatol 2019;81:520-6.)

Key words: basal cell carcinoma; melanoma; Delphi consensus method; diagnostic criteria; nevi; reflectance confocal microscopy; squamous cell carcinoma.

Reflectance confocal microscopy (RCM) allows noninvasive, in vivo examination of the skin at near-histopathologic resolution. RCM enhances the accuracy of skin cancer diagnosis compared with clinical and dermoscopic diagnosis.[1-5] The 2007 consensus paper on the RCM

terminology for melanocytic neoplasms lists more than 50 RCM terms.[6] Since then, additional RCM descriptors for melanocytic and nonmelanocytic neoplasms have been published.[7,8]

Diagnostic expertise entails prolonged training, accumulation of personal experience, and exposure to peers' cases. Indeed, interpretation of the RCM pattern of skin lesions requires explicit skill rather than direct translation of prior proficiency in histopathologic or dermoscopic diagnosis; RCM readers need to adapt to making diagnoses based on grayscale, horizontally oriented RCM images and based on recognition of key criteria from the extensive list of published RCM features. Presently, there are few basic courses on RCM, extending from hours to few days; comprehensive training programs are not readily available, and academic programs using RCM are scant.

CAPSULE SUMMARY

- Via expert consensus, we identified 4 reflectance confocal microscopy key features for skin cancer diagnosis.

- Novice users of reflectance confocal microscopy may diagnose common skin cancers with reasonable sensitivity and specificity by recognizing a short list of quintessential confocal features. This simplified approach may facilitate dissemination of confocal among dermatologists.

Given the constraints of contemporary RCM training and in the face of amassing RCM literature, we reasoned that identifying a short list of the most fundamental key criteria for skin cancers diagnosis may facilitate RCM training of novices and dissemination into clinical practice. To that end, we aimed to identify, via a modified Delphi expert consensus, the RCM key features of skin cancers and test their diagnostic utility for skin cancer diagnosis by novice readers.

MATERIAL AND METHODS

The local institutional review board approved this retrospective study.

Identification of RCM key features through modified Delphi experts' consensus

The first phase consisted of an extensive RCM literature review for melanoma, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC) diagnosis. A list of 56 published RCM features were identified via literature review.

We conducted expert consensus via a modified Delphi method.[9] A total of 6 experts (G.P., A.S., S.G., P.G., J.M., and C.L.) were selected on the basis of number of publications and years of expertise in diagnosis of melanoma and nonmelanoma skin cancer (NMSC). Each expert was asked to score the importance and relevance for melanoma diagnosis

of each of the 56 RCM features; the scale ranged from 0 (useless and completely irrelevant for melanoma diagnosis) to 10 (highly important for diagnosis or for ruling out melanoma with high confidence). When a feature was evaluated as useful for ruling out melanoma diagnosis and assigned a score 7 or higher, the expert was asked which specific diagnosis was indicated by the presence of this feature.

The resultant list of "valued terms" consisted of the terms that received an overall mean score (based on the scores of all 6 readers) of 7 or higher OR a score of 7 or higher in the individual evaluation of at least 4 of the 6 experts. We then created a table of definitions for these terms based on the literature and condensed the list to few cytologic and architectural key terms for melanoma, BCC, and SCC, with comprehensive definitions that synthesize and encompass multiple individual terms. We circulated these key terms with a draft of suggested comprehensive definitions among the experts and asked whether they agreed with the synthesis and with the proposed definitions. The experts could edit and suggest their version for the definitions. All the experts approved the final list of RCM terms.

Diagnostic utility study of the key RCM features

The diagnostic utility of the RCM terms was evaluated by testing the diagnostic accuracy of novice RCM readers on a retrospective image data set.

Novice reader training. A total of 10 dermatology residents participated in a 5-day training course focusing on RCM diagnosis of skin cancer at a tertiary medical center specializing in RCM imaging. Of the 5 days, 2 consisted of frontal lectures with particular focus on the key diagnostic features identified in the Delphi consensus and 3 included exposure to real-life cases (whereby RCM was integrated in clinical decision making), as well as retrospective case presentations. Additionally, each trainee evaluated online a retrospective series of 100 RCM cases (10 weekly cases over 10 weeks). Trainees were asked to identify RCM features on a standardized evaluation form. The evaluation of an RCM expert of the same cases was used to give

feedback to trainees on their accuracy in RCM feature identification.

Validation study. The study was performed on a retrospective data set of 1000 consecutive cases for which RCM imaging was integrated into skin cancer diagnosis between January 2010 and August 2011 at UNIMORE. RCM images were obtained by using the Vivascope 1500 reflectance confocal imaging system (MAVIG GmbH, Munich, Germany), as previously described.[7] A minimum of 3 mosaics—0.5 × 0.5-mm RCM images acquired and stitched into larger composite images—were obtained at the spinous-granular layers, dermoepidermal junction (DEJ), and papillary dermis. Video mode RCM images were not provided. "Ground truth" diagnoses were either histopathologically determined or, for cases in which no biopsy had been performed, based on clinical, dermoscopic, and RCM imaging, as well as stability upon long-term (>1 year) digital dermoscopic monitoring. The biopsy-proven diagnoses included 176 malignant cases, including 83 melanomas (mean Breslow thickness, 0.54 mm; standard deviation, 0.69 mm), 87 BCCs, and 6 SCCs. The remaining 824 cases included 749 nevi (of which 16 were Spitz nevi), solar lentigo (SL)/seborrheic keratosis (SK)/lichen planus—like keratosis (LPLK) (n = 57), and other benign nonmelanocytic lesions (eg, dermatofibromas and angiomas [n = 18]). Witkowski et al[7] previously used this 1000-case data set for a retrospective study; however, the novice readers were not shown these cases while attending the course or during the subsequent online training.

The data set was randomly divided into 100-case subsets among the 10 novice readers. The images were available for online reading over a 2-months period. For each case, readers evaluated the presence or absence of each of the 4 key RCM features identified via the Delphi consensus. Evaluations of RCM images were blinded to patients' demographic or clinical data and to the clinical and dermoscopic images.

Statistics

Statistical analyses were performed by using SPSS Statistics Professional software (version 20.0.0, IBM, Armonk, NY). For the modified Delphi analysis, mean and standard deviation, median, mode, and interquartile ranges of scores assigned by the 6 experts to each feature were calculated. For RCM cases, absolute number and frequency of RCM key features, as reported by the reader, were measured against the ground truth diagnosis. We considered an RCM reading of a lesion to be positive for skin cancer diagnosis if the reader identified at least 1 of the 4 RCM key features. Overall sensitivity (number of skin cancers correctly diagnosed) and specificity (number of benign lesions correctly diagnosed) were calculated. Sensitivity for melanoma, BCC, and SCC and specificity for benign diagnostic entities were also calculated separately.

RESULTS

Delphi consensus on RCM key features for melanoma and NMSC diagnosis

From 56 features, the expert panel considered 18 (32.1%) to be valued terms for skin cancer diagnosis (Table I). Most of the features (15 of 18 [83.3%]) were considered relevant for melanoma diagnosis, whereas the remaining 3 features were pertinent for the diagnosis of BCC (n = 2) and SCC (n = 1). Notably, honeycomb pattern marginally attained consensus threshold, with 4 of 6 experts assigning a score 7 or higher but with a mean score lower than 7. Of the remaining 38 features, 5 fell short of the consensus threshold, with only 3 of 6 experts assigning a score of 7 or higher and with a mean score lower than 7 (Table I). The other 33 features were well below the consensus threshold for skin cancer diagnosis.

On the basis of the definitions in the literature, the valued RCM features were grouped into (1) melanoma-specific features, including cytologic and architectural features, and (2) BCC- and SCC-specific features. On the basis of synthesis of these 18 RCM features, 4 comprehensive key features (2 for melanoma, 1 for BCC, and 1 for SCC) were defined, and these definitions were further revised on the basis of expert panel review.

The consensus key RCM definitions follow.

Atypical cells. Defined as large (>20-μm), roundish and/or dendritic cells in the suprabasal epidermis and/or DEJ and/or dermis, either presenting as single isolated cells or forming clusters (Fig 1). The suspicion for melanoma increases with a cellular density greater than 5 per mm^2, with cellular pleomorphism, with large and morphologically unusual shapes (triangular or star-shaped), and with atypical cells in pagetoid spread that are roundish, diffuse, or reaching high in the suprabasal epidermis. Exceptions include the presence of large round but

Table I. RCM features recognized via expert Delphi consensus as valuable for skin cancer diagnosis

Feature	Experts assigning score ≥7, n	Mean score	Associated diagnosis
Feature attaining consensus threshold*			
Dermal papillae attributes: contour (edged/nonedged)	5	7.5	Melanoma
Dermoepidermal junction disarray (mild/moderate/severe)	5	7.7	Melanoma
Nest (cluster): types	5	8.5	Melanoma
Cellular atypia	5	8.8	Melanoma
Cellular monomorphism	5	7.7	Melanoma
Cellular pleomorphism	5	8.3	Melanoma
Cell shape	5	7.2	Melanoma
Cell size	5	7.0	Melanoma
Pagetoid spread (present/absent)	5	8.7	Melanoma
Pagetoid cells: shape	5	8.7	Melanoma
Pagetoid cells: size	5	8.0	Melanoma
Pagetoid cells: density	5	8.3	Melanoma
Pagetoid cells: distribution	5	7.7	Melanoma
Pagetoid cells: extension to stratum corneum	5	8.0	Melanoma
Pagetoid cells: pleomorphism	5	8.3	Melanoma
Basaloid cords/islands	5	9.2	Basal cell carcinoma
Clefting	5	7.8	Basal cell carcinoma
Honeycomb pattern	4	5.3	Squamous cell carcinoma
Feature falling short of consensus threshold[†]			
Dendrites: presence	3	5.7	Melanoma
Pagetoid cells: aggregation	3	5.5	Melanoma
Basal layer pattern: uniformity (uniform/nonuniform)	3	4.8	Melanoma
Fibrillar polarized pattern around tumor	3	6.3	Basal cell carcinoma
Epidermal shadow	3	5.2	Basal cell carcinoma

RCM, Reflectance confocal microscopy.
*Between 4 and 6 evaluators assigned the feature a score 7 or higher OR the mean Delphi score for the feature was 7 or higher.
[†]Of the 6 evaluators, 3 assigned the feature a score 7 or higher, with a mean Delphi score for the feature less than 7.

monomorphic cells in junctional or dermal nests in the context of a regular clod pattern; and atypical cells, either roundish or dendritic, inside basaloid cords/islands or in the overlying epidermis in BCC.

DEJ disarray. Architectural disorder at the DEJ includes nonedged papillae (Fig 1), disorganized/chaotic pattern (ringed, meshwork, and/or clod patterns that are asymmetrically distributed or irregular in appearance), or poorly defined areas. DEJ disarray should encompass more than 10% of the lesion area. "Poorly defined area" is an area that lacks any of the recognizable patterns associated with melanocytic lesions (ringed, meshwork, and clod patterns) or with nonmelanocytic lesions (ringed pattern, polycyclic papillary contours, enlarged interpapillary spaces, central scar with tethered peripheral rings, lacunas, basaloid cords/island, or dark silhouettes).

Basaloid cords/islands. These correspond to (1) roundish, elongated, or polycyclic refractive structures with cellular palisading at the periphery (Fig 2), frequently outlined by a "dark cleft" that separates the refractive structure from the surrounding, usually bright stroma, or to (2) dark homogenous areas with lobulated contours embedded in bright fibrotic stroma ("dark silhouettes").

Keratinocyte disarray. Irregular honeycomb pattern of the epidermis (lines of honeycomb that vary in thickness and brightness, angulated cells, and pleomorphic nuclei of keratinocytes [Fig 3]) or lack of a recognizable pattern (ie, absence of honeycomb or cobblestone) of the epidermis.

Clinical validation study

The evaluation of 100 cases by each of the 10 novice readers was compared with the ground truth diagnosis (Table II). Melanoma-specific features (ie, atypical cells and DEJ disarray) were identified in 66 (79%) and 74 (89%) of the 83 melanomas, respectively. Although Spitz nevi also frequently showed atypical cells or DEJ disarray (81% and 56% of the evaluations, respectively), these features were observed with lower frequency (166 [23%] and 221 [30%]) among the non-Spitz nevi, and very rarely among NMSC or benign nonmelanocytic lesions. Basaloid cords/islands were observed almost
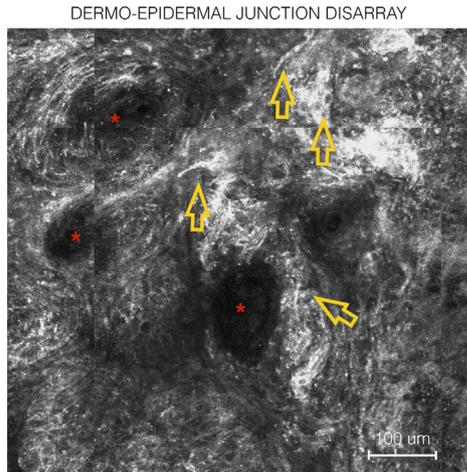
DERMO-EPIDERMAL JUNCTION DISARRAY

Fig 1. Melanoma. An optical section (0.5 × 0.5 mm) showing dermoepidermal junction disarray. The dermal papillae (*asterisks*) are nonedged because they lack a bright demarcating rim and are separated by broadened interpapillary spaces (widened rete ridges). There is a disorganized junctional infiltration of melanocytes as large dendritic atypical cells and as poorly demarcated bright nests (*arrows*).
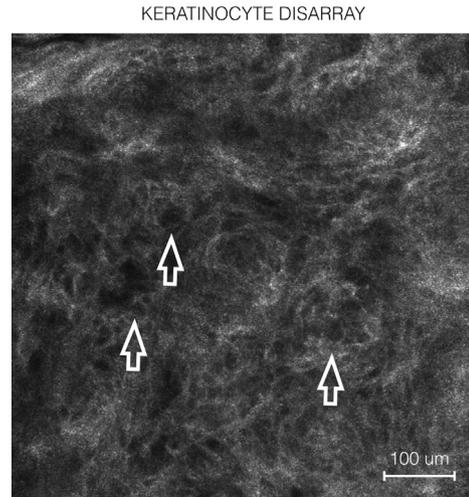
BASALOID ISLANDS/CORDS

Fig 2. Basal cell carcinoma. An optical section (0.5 × 0.5 mm) at the superficial dermis level showing basaloid islands/cords. There are bright roundish aggregates (*asterisks*) with peripheral palisading of nuclei (*yellow arrow*) separated by dark clefts from the dermal stroma that shows *en face* dilated blood vessels (*white arrow*) and infiltrate of melanophages (*blue arrows*).

exclusively in BCC (73 of 87 [84%]), and rarely in other diagnoses. Keratinocyte disarray was observed in 50% of SCCs, in 12 melanomas (14%) and in 17 BCCs (19%), as well as in 7 of 57 SL/SK/LPLK (12%). Considering the presence of at least 1 of the 4 key features, the sensitivity for skin cancer was 91%

KERATINOCYTE DISARRAY

Fig 3. Squamous cell carcinoma. An optical section (0.5 × 0.5 mm) at the level of the spinous-granular layers of the epidermis showing keratinocyte disarray. There is an irregular honeycomb pattern of the epidermis with variability in thickness and brightness of the honeycomb's lines and in the size of the nuclei (*arrows*).
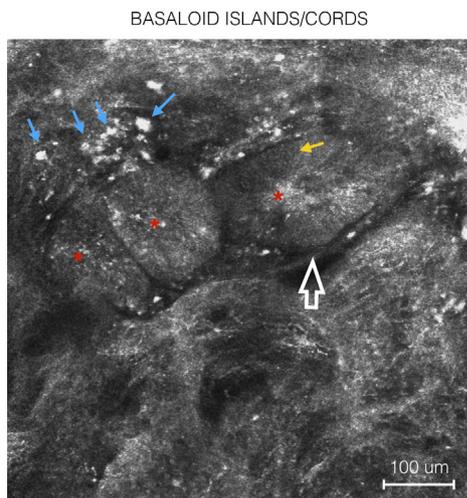
(93% for melanoma, 92% for BCC, and 67% for SCC), whereas the specificity was 57% (56% for nevi other than Spitz; 12% for Spitz nevi; 77% for SL/SK/ LPLK; and 94% for other benign nonmelanocytic lesions).

DISCUSSION

RCM has opened up a new era of "optical biopsies," with an evident application to skin cancer diagnosis given the high reflective index of melanin and keratin. The mosaicking of consecutive RCM images into a larger field of view and the *en face* plane of RCM imaging allow direct correlation between dermoscopic patterns and cytologic and architectural findings under RCM,[10-12] and the integration of dermoscopy and RCM in the diagnosis of melanoma and NMSC.[3,4,13] Recently, studies testing the utility of combining RCM with digital dermoscopic monitoring have demonstrated a reduction in the number of lesions needed to excise to diagnose skin cancer, reflecting an approximately 2-fold reduction in unnecessary biopsies of benign lesions.[5,14,15]

Despite the evident utility of RCM for skin cancer diagnosis and management, the diffusion of this technology into clinical practice has been slow and limited, and mostly centered around academic medical centers. One reason could be the substantial cost of RCM. However, another barrier to adoption may lie in the need for specific training to attain RCM diagnostic proficiency. Recently, Witkowski et al

Table II. Identification of each of the RCM key features by diagnosis in the study data set (N = 1000)

Diagnosis (No. of lesions)	RCM key features				
	Atypical cells, n (%)	DEJ disarray, n (%)	Basaloid cords/ islands, n (%)	Keratinocyte disarray, n (%)	Number of RCM-positive lesions, n (%)*
Melanoma (n = 83)	66 (79.5%)	74 (89.2%)	3 (3.6%)	12 (14.5%)	77 (92.8%)
Basal cell carcinoma (n = 87)	2 (2.3%)	6 (6.9%)	73 (83.9%)	17 (19.5%)	80 (91.9%)
Squamous cell carcinoma (n = 6)	0 (0%)	0 (0%)	1 (16.7%)	3 (50%)	4 (66.7%)
Nevi (excluding Spitz) (n = 733)	166 (22.6%)	221 (30.2%)	2 (0.3%)	8 (1.1%)	323 (44.1%)
Spitz nevi (16)	13 (81.2%)	9 (56.2%)	0 (0%)	1 (6.2%)	14 (87.5%)
SL/SK/LPLK (n = 57)	7 (12.3%)	1 (1.8%)	1 (1.8%)	7 (12.3%)	13 (22.8%)
Other benign nonn-melanocytic lesions (n = 18)	0 (0%)	0 (0%)	1 (5.6%)	1 (5.6%)	1 (5.6%)

Each of the 10 novice readers evaluated a subset of 100 lesions, for a total data set of 1000 lesions.
LPLK, Lichen planus–like keratosis; *RCM*, reflectance confocal microscopy; *SK*, seborrheic keratosis; *SL*, solar lentigo.
*RCM-positive lesions are those showing at least 1 RCM key feature.

showed that good diagnostic accuracy can be achieved by RCM novices, following a 6-month training program with exposure to more than 4000 cases; sensitivity can be as high as 98% by the use of "double reading" (ie, consensual diagnosis of each real-life case by 2 readers, typically a novice and an experienced RCM reader).[7]

Herein we have focused on differentiating melanoma, BCC, and SCC from their most common dermoscopy mimickers, such as nevi and benign nonmelanocytic lesions. Our aims were to simplify terminology to a few key terms by using expert consensus via a modified Delphi methodology, and to test the diagnostic utility of this simplified approach among novice trainees.

A panel of 6 RCM experts identified 18 RCM terms as the most useful for diagnosis of skin cancer. By grouping related terms, 4 key terms—namely, atypical cells, DEJ disarray, basaloid cords/islands, and keratinocyte disarray—were extracted and defined in a manner that integrates and unifies the 18 RCM terms.

For the diagnostic utility study, the novice readers were not asked to provide a diagnosis or management decision; rather, they were asked only to recognize the presence or absence of each of the 4 key features. The 91% overall sensitivity and 57% overall specificity attained by the readers are comparable to the accuracy levels in previous publications wherein diagnosis was based solely on RCM imaging.[2-4,16] In another previous study[7] using the same RCM data set as the present study, readers received longer training, utilized a comprehensive list of RCM features for diagnosis, and were provided with dermoscopic images; the mean sensitivity and specificity for individual readers were 95% and 76%, respectively, whereas the "double readers"

sensitivity and specificity were nearly 99% and 66%, respectively.

Hence, the simplified approach presented herein may facilitate the adoption of RCM by novice users. A broader diffusion of RCM into clinical practice has become increasingly likely following the allotment of category I Current Procedural Terminology reimbursement codes for RCM examination by the Centers for Medicare and Medicaid Services in the United States.[17] The current study suggests that with simplified terminology, identification of the most relevant features, and a short focused teaching program, novices can rapidly acquire the essential tools for the immediate clinical application of RCM. That said, until a high level of experience and confidence are achieved, novice clinicians should interpret the results of RCM examination in the context of their clinical and dermoscopic diagnosis. The RCM interpretation of the novice should guide clinical management only when unequivocal findings are seen or when RCM confirms the clinical diagnosis. Clinical dermoscopic diagnosis should trump RCM interpretation when there is discrepancy in the face of high clinical dermoscopic suspicion of skin cancer or when RCM findings are equivocal.

Our study has limitations. First, it is a retrospective study, and thus the 4 key features have to be tested prospectively and in real clinical setting. Second, a larger data set of cases is needed to further explore possible shortcomings of the key features for challenging lesions. Third, evaluations of RCM images were blinded to patients' demographic or clinical data and to clinical and dermoscopic images; these data may influence, and possibly enhance, their diagnostic accuracy. Fourth, the Delphi consensus was based on only 6 RCM experts, albeit from different institutions and countries. Fifth, although

the readers were novice RCM users, they received comprehensive on-site and on-line training; their performance may not represent that of novices with shorter training. Finally, an experienced RCM technician acquired the reader study images; in real life, novices' image interpretation may be limited by suboptimal image acquisition during the initial imaging technique learning curve period.

In conclusion, the simplified approach presented herein may enhance novice RCM readers' accuracy for skin cancer diagnosis and facilitate the diffusion of RCM to dermatology clinics beyond academic centers.

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