Reflectance confocal microscopy

Principles, basic terminology, clinical indications, limitations, and practical considerations

Neda Shahriari, MD,[a] Jane M. Grant-Kels, MD,[a,b] Harold Rabinovitz, MD,[c,d] Margaret Oliviero, NP,[c] and Alon Scope, MD[e,f]

Farmington, Connecticut; Gainesville and Plantation, Florida; Augusta, Georgia; Tel Aviv, Israel; and New York, New York

Learning objectives

After completing this learning activity, participants should be able to describe the reflectance confocal microscopy (RCM) features of normal skin by anatomic layer of the skin utilizing basic terminology; recognize clinical indications and limitations in the use of RCM, including diagnostic sensitivity and specificity, pre-surgical cancer margin mapping, recognition of tumor recurrence at surgical sites, and monitoring ablative and non-invasive therapy and discuss practical considerations including logistics of adding RCM to practice, learning curve in image acquisition and interpretation and appropriate billing.

Disclosures

Dr Rabinowitz is a speaker for Caliber and has received equipment in the past. Ms Oliviero is a speaker and consultant for Caliber ID. Drs Shahriari, Grant-Kels, and Scope have no conflicts of interest to declare.

Editors

The editors involved with this CME activity and all content validation/peer reviewers of the journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

Authors

The authors involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

Planners

The planners involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s). The editorial and education staff involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

Reflectance confocal microscopy (RCM) is a noninvasive imaging tool used for in vivo visualization of the skin. It has been extensively studied for use in the evaluation of equivocal cutaneous neoplasms to decrease the number of biopsy procedures in patients with benign lesions. Furthermore, its applications are broadening to include presurgical cancer margin mapping, tumor recurrence surveillance, monitoring of ablative and noninvasive therapies, and stratification of inflammatory disorders. With the approval of category I *Current Procedural Terminology* reimbursement codes for RCM image acquisition and interpretation, use of this technology has been increasingly adopted by dermatologists. The first article in this 2-part continuing medical education series highlights basic terminology, principles, clinical

From the Department of Dermatology,[a] University of Connecticut Health Center, Farmington; Department of Dermatology,[b] University of Florida, Gainesville; Skin and Cancer Associates,[c] Plantation; Dermatology Department,[d] Medical College of Georgia at Augusta University, Augusta; The Kittner Skin Cancer Screening and Research Institute,[e] Sheba Medical Center and Sackler Faculty of Medicine, Tel Aviv University; and the Dermatology Service,[f] Memorial Sloan-Kettering Center, New York.

Supported by the Israel Science Foundation (ISF-1546-16).

Dr Rabinowitz is a speaker for Caliber and has received equipment in the past. Ms Oliviero is a speaker and consultant for Caliber ID. Drs Shahriari, Grant-Kels, and Scope have no conflicts of interest to declare.

Accepted for publication May 12, 2020.

Reprint requests: Neda Shahriari, MD, Department of Dermatology, University of Connecticut Health Center, 21 South Rd, Farmington, CT 06032. E-mail: shahriari@uchc.edu.

0190-9622/$36.00

https://doi.org/10.1016/j.jaad.2020.05.153

Date of release: January 2021.
Expiration date: January 2024.

Scanning this QR code will direct you to the CME quiz in the American Academy of Dermatology's (AAD) online learning center where after taking the quiz and successfully passing it, you may claim *1 AMA PRA Category 1 credit*. NOTE: You must have an AAD account and be signed in on your device in order to be directed to the CME quiz. If you do not have an AAD account, you will need to create one. To create an AAD account: go to the AAD's website: www.aad.org.

applications, limitations, and practical considerations in the clinical use of RCM technology. (J Am Acad Dermatol 2021;84:1-14.)

Key words: basic terminology; billing; logistics; optical principles; presurgical planning; reflectance confocal microscopy; specificity and sensitivity; tumor recurrence surveillance.

The evaluation of skin lesions has evolved from naked-eye inspection to the incorporation of dermoscopy. While dermoscopy has increased our diagnostic acumen,[1,2] a continued shared goal among dermatologists is to improve the diagnosis of malignant lesions while minimizing the number of unnecessary biopsy procedures in patients with benign neoplasms. Among adjunct noninvasive tools to further this objective is reflectance confocal microscopy (RCM). RCM enables the capture of in vivo, cellular-resolution images of lesions, parallel to the skin surface, at different depths from the stratum corneum to the superficial dermis. Given the approval of *Current Procedural Terminology* codes for the use of RCM, there has been an increasing utilization rate by dermatologists. However, there is a knowledge gap regarding the optical principles, basic terminology, clinical indications, and limitations in the use of RCM. These are addressed in the first article in this continuing medical education series.

BASIC OPTICAL AND IMAGE ACQUISITION PRINCIPLES

The light source used in RCM is a near-infrared low-power laser (830-nm diode, power <35 mW) that emits monochromatic coherent light.[3-5] The initial path of light from the source to the tissue is through a system of optical lenses and mirrors (Fig 1).[5] The light scans a focal point in the tissue. The subsequently reflected ("back scattered") light from the tissue passes through a gating pinhole to the detector—only light from the focal area imaged can enter through the pinhole, while "out of focus" scattered light is blocked. RCM images appear in grayscale and are based on relative refractive indices of tissue elements—those with a higher refractive index appear brighter. Melanin, for example, has high refractive index of 1.7, and therefore cells containing melanin appear brighter than surrounding tissue elements.[6] Sample refractive indices are shown in Table I. The basic RCM image is a single "optical section" that instantaneously displays a 0.5- × 0.5-mm^2 field of view at the horizontal (X-Y) plane. By moving the objective lens toward the skin, the focal plane is moved progressively deeper (on the Z-axis).[5]

There are 2 types of RCM devices: the wide-probe and handheld (Fig 2).

Wide-probe RCM requires fixation of the probe to the skin[4,5] and is therefore limited to flat surfaces (e.g., the cheek). A metal tissue ring is affixed to the skin with an adhesive and disposable polycarbonate window. A drop of immersion fluid (commonly mineral oil) is placed on the skin before applying the ring—window assembly and a water-based gel is applied to the objective lens as another immersion medium.[5] The RCM probe is then magnetically coupled to the tissue ring. The wide-probe RCM device simulates a shave biopsy procedure. It allows stitching together of 16 × 16 adjacent optical sections at the same image depth, creating mosaic images up to 8- × 8-mm^2 in field of view.[4] Appropriately sized mosaics attempt to encompass the entire lesion with rims of normal skin. If the lesion diameter is >8 mm, the ring is centered on the most suspicious area; if heterogeneously colored and large, the ring can be placed so that images sample disparate areas. The user can digitally control probe movement in the horizontal plane and on the Z-axis. From the skin surface to the superficial dermis, stacks of individual optical sections of the same X-Y area of skin can be automatically captured.

On the other hand, the handheld device has a smaller probe that does not require fixation to the skin. After applying a drop of immersion oil to the surface, movement in the X-Y plane depends on manually sliding the RCM probe on the skin. The handheld RCM therefore enables imaging of curved anatomic sites (e.g., ears). While automated image stacks can be acquired with the handheld device, mosaic images cannot be stitched together, so that its field of view is limited to 0.75 × 0.75 mm^2.

RCM OF NORMAL SKIN

Skin surface/corneal layer

The stratum corneum appears bright because of the high refractive index of keratin (Fig 3). Linear dark fissures, representing the dermatoglyphs, separate "islands" of keratinocytes.[7,8] The corneocytes measure 10 μm to 30 μm and appear as flat, polygonal anucleate cells.[9] Thickness of the stratum

Abbreviations used:

BCC: basal cell carcinoma
DEJ: dermoepidermal junction
LM: lentigo maligna
NNE: number needed to excise
OCT: optical coherence tomography
RCM: reflectance confocal microscopy
SCC: squamous cell carcinoma

corneum ranges from 0 μm to 15 μm, depending on the anatomic location.

Granular layer

The granular layer is located 15 μm to 20 μm below the surface.[9] It consists of viable keratinocytes with central dark nuclei and surrounding refractile granular cytoplasm resulting from organelles and keratohyalin granules.[8-10] This layer typically has a "honeycomb" pattern resulting from the back-to-back arrangement of keratinocytes that display polygonal bright cellular outlines.[7]

Spinous layer

The spinous layer is located at a depth of 20 μm to 100 μm and has a higher keratinocytic density than the granular layer.[8,9] Keratinocytes decrease in size with deeper layers. The spinous layer also forms a honeycomb pattern, consisting of bright polygonal keratinocytes with refractile cytoplasm and dark central nucleus (Fig 4).

Basal layer and the dermoepidermal junction

At a depth of 50 μm to 100 μm, the basal keratinocytes often display an opposite refractivity pattern: cells are brighter at the center than at the peripheral cytoplasm because melanin caps encase the nuclei. This yields a cobblestone pattern formed by clusters of numerous bright round basal keratinocytes above the dermal papillae (Fig 5).[5,6] The cobblestone pattern is more pronounced in individuals with darker skin.[7,9] Within the basal layer, melanocytes may be identified; they do not always take on the classic dendritic form, but rather can have varied shapes, including oval and fusiform.[11]

At the dermoepidermal junction (DEJ) there are oval to round "rings" composed of brighter circumferentially arranged basal keratinocytes around darker, less refractile dermal papillae; this feature is referred to as "edged papillae" (Fig 6). When capturing the image, blood vessels running through the dermal papillae can be visualized in real-time.

Superficial dermis

At a depth of 100 μm to 150 μm, collagen fibers forming a reticulated pattern can be seen.[8,9] Dermis that harbors fibrosis or solar elastosis shows more refractile collagen than normal skin. In the deeper reticular dermis, collagen appears as parallel-arranged thicker bundles. Melanophages may appear as plump, irregularly shaped stellate highly refractile cells without a visible nucleus.[7,10] Only

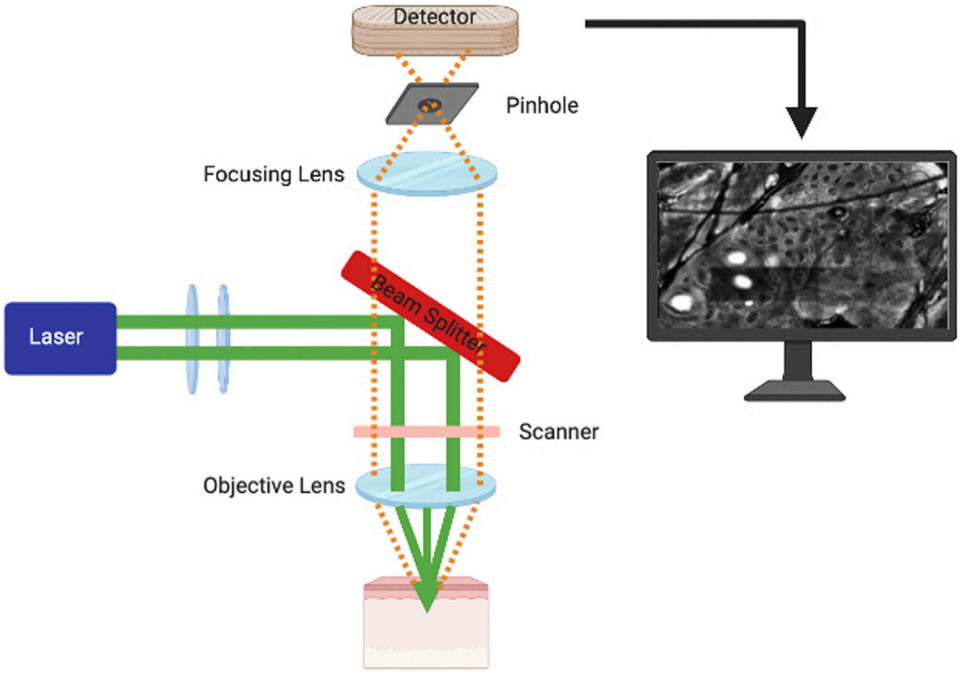

Fig 1. Diagram depicting optical principles of reflectance confocal microscopy.

Table I. Refractive indices of different components of the skin in descending order

Skin components	Refractive index
Melanin in pigmented keratinocytes or melanocytes[6]	1.7
Keratin[74]	1.5
Hair[75]	1.37-1.7 (depending on melanin content)
Stratum corneum[4]	1.55
Collagen, radial[76,77]	1.40-1.61
Organelles, protein fibrils, membranes, and protein globules[77]	1.39-1.47
Dermis[78]	1.36-1.41
Intercellular liquid and cytoplasm, ground substance[77]	1.35-1.37
Epidermis[4]	1.34
Collagen, axial[76,77]	1.32-1.45
Red blood cells[79]	1.40-1.42
Inflammatory cells[80]	1.38-1.48
Serum and plasma[81]	1.39
Nuclei[82]	1.23-1.34

superficial components of adnexal structures can be seen by RCM.

RCM BASIC TERMINOLOGY

Key terms and patterns used to describe RCM features in the skin are described in Table II.

CLINICAL INDICATIONS

Diagnosis of cutaneous neoplasms

RCM should not replace but should instead serve as an adjunct to clinical and dermoscopic examination.[12] At present, the main clinical application of RCM is diagnosis of equivocal skin lesions with a low-to-moderate pretest probability of malignancy. RCM is not aimed at diagnosis of lesions with clear-cut criteria for malignancy based on clinical and dermoscopic examination. In particular, RCM is worthwhile in instances where clinicians are more hesitant to obtain a biopsy specimen—adults with lesions in cosmetically sensitive areas (e.g., the face) or regions with poor wound healing (e.g., the lower extremities).

Borsari et al[13] found in a retrospective analysis that one of the best indications for RCM is diagnosis of lesions located on sun-damaged skin, particularly the head and neck areas. In our experience, there are 2 indications that are highly amenable to RCM imaging. First, pigmented macules/patches on sun-damaged skin with differential diagnosis of clinically atypical solar lentigo/flat seborrheic keratosis, lichen planus–like keratosis, pigmented solar keratosis, and lentigo maligna (LM). Second, nonpigmented facial papules; the differential diagnosis ranges from benign lesions (intradermal nevus or angiofibroma) to skin cancers (basal cell carcinoma [BCC] or squamous cell carcinoma [SCC]). In both indications, diagnostic entities usually show different RCM criteria and accurate diagnosis leads to different management.

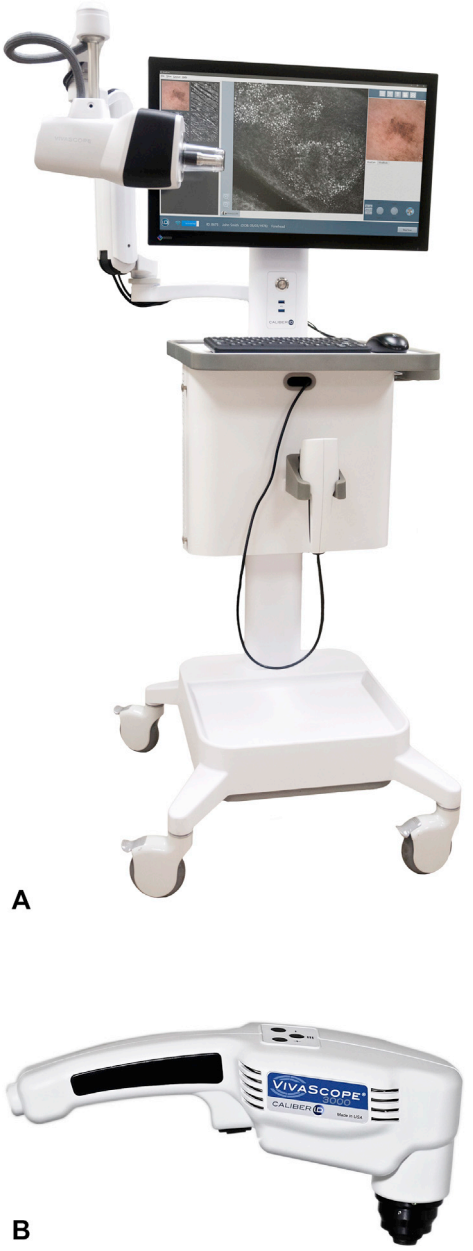

Fig 2. Reflectance confocal microscopy (RCM) devices. **A**, A wide-probe RCM device (Vivascope1500). **B**, A handheld RCM device (Vivascope 3000). Courtesy of Caliber I.D., Andover, MA.

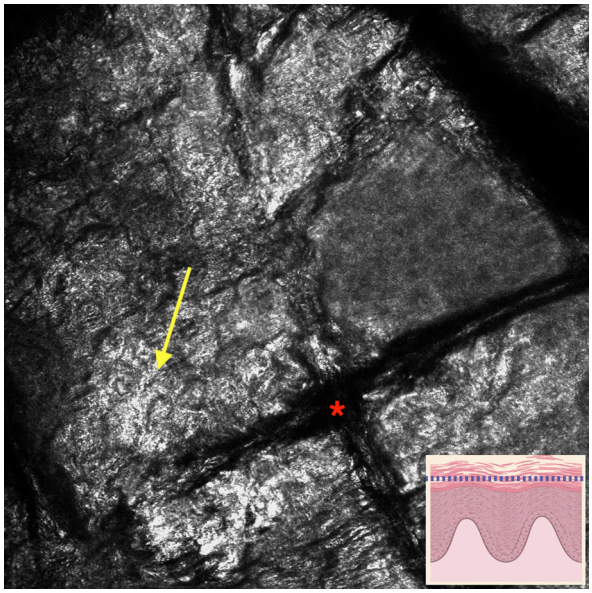

Fig 3. Normal skin. Reflectance confocal microscopy optical section (0.5 × 0.5 mm^2) at the level of the stratum corneum showing a bright layer of flattened anucleate keratinocytes (yellow arrow), interrupted by dark fissures (red asterisk) representing dermatoglyphs.

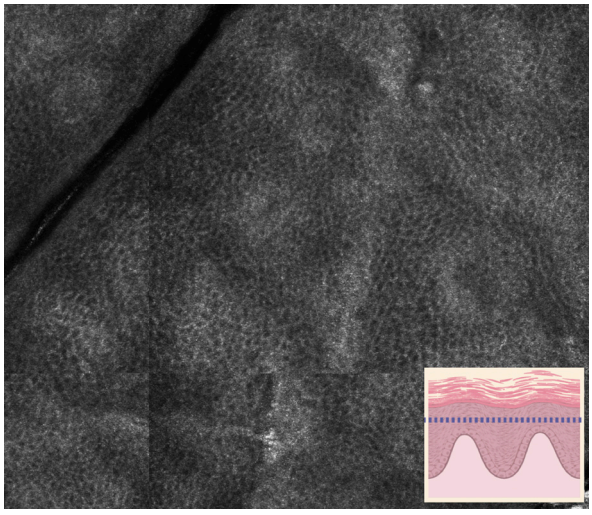

Fig 4. Normal skin. Reflectance confocal microscopy mosaic (1.5 × 1.5 mm^2) at the level of the mid-spinous layer showing uniformly sized and spaced keratinocytes. The central nucleus of keratinocytes appears dark, while the surrounding cytoplasm presents well-demarcated bright polygonal outlines, forming a honeycomb pattern.

Several studies have shown that RCM increases diagnostic accuracy (Table III). For melanoma diagnosis with RCM, a systematic review pooling data from 5 studies comprising 909 lesions found a sensitivity of 93% (95% confidence interval [CI] 89-96%) and specificity of 76% (95% CI 68-83%).[14] A metaanalysis of RCM for BCC diagnosis found a

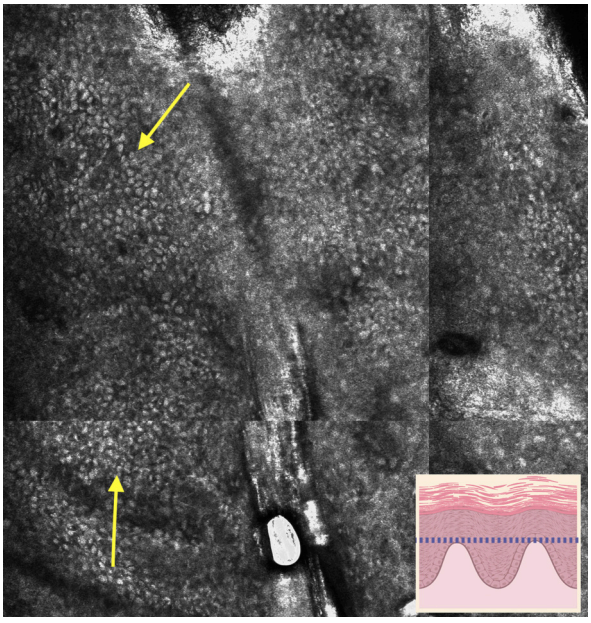

Fig 5. Normal skin. Reflectance confocal microscopy mosaic (1 × 1 mm^2) at the basal layer depicting closely set cuboidal to round bright keratinocytes (arrows) forming a cobblestone pattern.

sensitivity of 97% (95% CI 90-99%) and specificity of 93% (95% CI 88-96%).[15] In our experience, RCM allows in most cases to confirm a suspicion of BCC and enables the clinician to proceed directly to definitive treatment. A pooled analysis of 25 studies established an overall RCM sensitivity and specificity of 79% to 100% and 78% to 100%, respectively, for SCC, SCC in situ, and keratoacanthoma.[16] For all skin cancer types, 1 metaanalysis pooling 21 studies (3108 patients, 3602 lesions) demonstrated overall sensitivity of 94% and specificity of 83%.[17] A prospective study found that RCM significantly increased clinicians' confidence in the diagnosis of dermoscopically equivocal lesions.[18]

Several studies underscore the utility of integrating RCM in high-risk pigmented lesion clinics where patients have multiple nevi. In one study, the number needed to excise (NNE) for the diagnosis of melanoma using dermoscopy alone was 14.6, while the use of RCM after dermoscopy reduced the NNE to 6.8 (a >50% reduction of unnecessary excisions).[19] Another study found that dermoscopy alone had a NNE of 3.7, while the combination of dermoscopy and RCM decreased NNE to 2.9, a significant difference.[20] In both studies, RCM-negative lesions were then followed with digital dermoscopy at 3 to 6 months and at 1 year to ensure stability.[19,20] Another study proposed to first perform digital dermoscopic monitoring of (flat) lesions for 3 months, followed by RCM imaging of lesions that

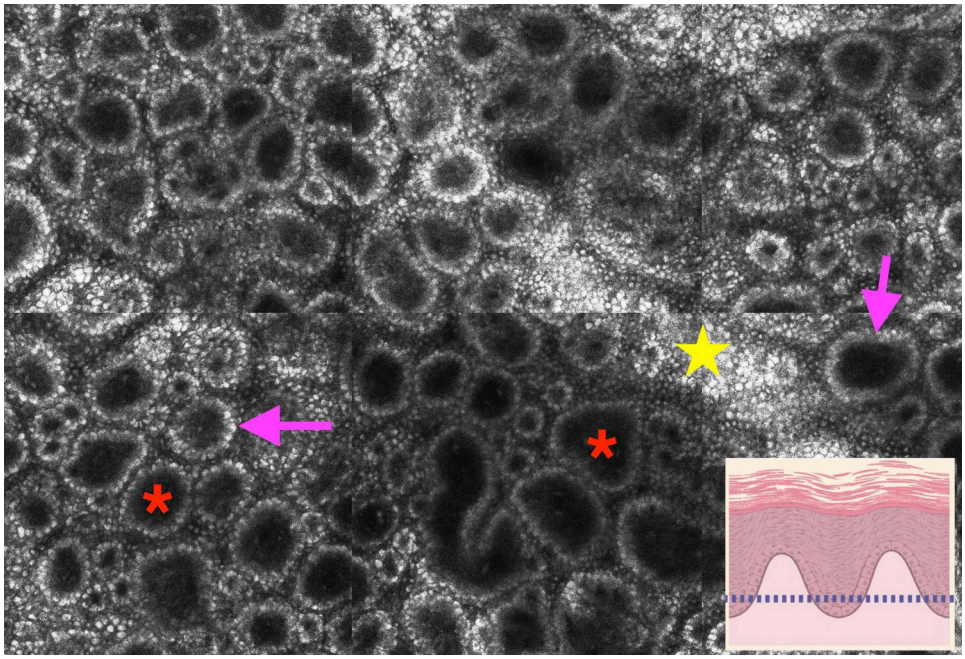

Fig 6. Normal skin. Reflectance confocal microscopy optical section (0.5 × 0.5 mm^2) at the level of the dermoepidermal junction showing dark dermal papillae (asterisks) with a well-demarcated rim of bright cells (arrow) forming a ringed pattern termed "edged papillae." This plane partially intersects the basal layer, and therefore the cobblestone pattern (star) can be visualized, interweaving amongst the edged papillae.

Table II. Basic relectance confocal microscopy terminology[7,9,74,83-85]

RCM pattern	RCM description	Histopathologic correlates
Epidermal layers		
Regular (typical) honeycomb	Polygonal bright cellular outlines of keratinocytes demonstrating uniformity in size and shape and in thickness and brightness of the lines	Normal pattern of keratinocytes in nonpigmented spinous and granular layers; brightness is caused by keratohyaline granules and organelles in the cytoplasm of keratinocytes[8]
Irregular (atypical) honeycomb	Polygonal bright cellular outlines of keratinocytes displaying variability in size and shape and nonuniformity in thickness and brightness of the lines	Atypical keratinocytes of the spinous and granular layers displaying disorganized arrangement and crowding and pleomorphism of nuclei
Broadened honeycomb	Polygonal outlines are thicker and brighter than usual, but overall uniform in size, shape, and brightness	Correlate not elucidated, but this RCM pattern is often associated with an acanthotic epidermis
Regular (typical) cobblestone	A uniform pattern of closely set, bright round cells separated by less refractile polygonal outlines	Keratinocytes are brighter at the center, because of melanin caps encasing nuclei, than at the peripheral cytoplasm; normal pattern of pigmented basal keratinocytes at suprapapillary plates, and variant of normal pattern of spinous-granular layers in darker skin
Irregular (atypical) cobblestone	A nonuniform pattern of closely set, bright round cells separated by less refractile polygonal outlines, with cells showing variability in size, spacing, and brightness	Atypical pigmented keratinocytes displaying disorganized arrangement and crowding and pleomorphism of nuclei

Continued

Table II. Cont'd

RCM pattern	RCM description	Histopathologic correlates
Disarranged epidermis	Epidermis that lacks recognizable honeycomb or cobblestone pattern.	Atypical keratinocytes displaying markedly disorganized arrangement
Pagetoid spread	Bright round or dendritic nucleated atypical cells in the suprabasal layers of the epidermis	Atypical melanocytes in the suprabasal layers; Langerhans cells may be difficult to distinguish from dendritic melanocytes
DEJ/papillary dermis		
Edged papillae	Dark dermal papillae surround by ring-like rim of bright cells	Normal pattern of undulating DEJ with dermal papillae surrounded by pigmented basal keratinocytes or melanocytes
Nonedged papillae	Dermal papillae without a demarcating bright rim of cells; adjacent dermal papillae may be separated by large reflecting cells	Irregular junctional proliferation of melanocytes resulting in disarrangement of undulating DEJ pattern
Ring pattern	Low-magnification DEJ pattern showing a predominance of "edged papillae"	Normal pattern of undulating DEJ with pigmented basal keratinocytes or melanocytes at the sides and tips of the rete ridges; seen also with lentiginous junctional proliferation of melanocytes
Meshwork pattern	Low-magnification DEJ pattern showing thickened, interconnecting, bright elongated aggregates that widen the interpapillary spaces	Elongated junctional nests of melanocytes along the sides and tips of the rete ridges, with bridging of adjacent retes
Clod pattern	Low-magnification superficial dermis pattern in which bright nests predominate	Large-nested proliferation in the superficial dermis
Composite pattern	Combination of the previous 3 RCM patterns (ring/meshwork/clod)	Combination of the above patterns
Dense nests	Round, oval, or polygonal sharply delineated compact cell aggregates	Round, oval, or polygonal junctional or dermal nests of melanocytes
Atypical cells	Large (>20 μm), roundish or dendritic nucleated cells in the suprabasal epidermis or DEJ or dermis, either presenting as single cells or forming nondense clusters	Atypical melanocytes
DEJ disarray	Architectural disorder including the presence of nonedged papillae, disorganized ring, meshwork, or clod patterns, or poorly defined areas that lack any recognizable pattern	An uneven undulating pattern or flattening of the DEJ, mostly in the context of neoplastic cellular proliferation
Basaloid cords/islands	Roundish, elongated, or polycyclic refractile structures with cellular palisading at the periphery, frequently outlined by a "dark cleft" that separates the refractive structure from the surrounding stroma	Refers to basaloid aggregates of basal cell carcinoma
Plump bright cells	Large bright cells in the dermis that are poorly demarcated and lack visible nucleus	Melanophages

DEJ, Dermoepidermal junction; *RCM*, reflectance confocal microscopy.

show minor or moderate dermoscopic change; the NNE was reduced by 47% through referral for excision only of lesions that show major changes in digital dermoscopic follow-up or that show RCM-positive features.[21]

Presurgical applications

Several studies have evaluated the potential role for RCM in presurgical margin evaluation of skin cancers to facilitate surgical planning. After obtaining a biopsy specimen, a clinical dermoscopic evaluation of BCC is unreliable in determining the presence of residual tumor versus complete clearance.[22-24] Among patients referred for Mohs micrographic surgery with biopsy-proven BCC but without clinical evidence of a residual tumor, RCM allowed presurgical detection of residual BCC with 92.8% sensitivity and 68.4% specificity—compared with frozen-section histopathology.[23] To compensate for the depth limitation of RCM, one prospective study

Table III. Overview of key studies evaluating the diagnostic accuracy of reflectance confocal microscopy

Study type	Lesions, N	Tumor evaluated	Accuracy
Metaanalysis[86]	498	LM	RCM sn: 93% RCM sp: 89% derm sn: 73% derm sp: 84%
Retrospective[51]	223	LM/LMM	RCM sn: 80% RCM sp: 81% derm sn: 61% derm sp: 92%
Metaanalysis[87]	1111	Amelanotic/hypomelanotic melanoma	RCM sn: 67% RCM sp: 89% derm sn: 61% derm sp: 90%
Prospective[88]	326	Melanoma	RCM sn: 91% RCM sp: 68% derm sn: 88% derm sp: 32%
Prospective[89]	117	Melanoma (n = 27)	RCM sn: 988.15% RCM sp: 97.60%
Cochrane metaanalysis[90]	2838	Melanoma (n = 658)	RCM sp: 82% derm sp: 42%
Retrospective[91]	64	Melanoma	RCM sn: 100% RCM sp: 69%
Prospective[92]	710	Melanoma (n = 216) BCC (n = 119)	RCM sn: 87.6% RCM sp: 70.8% RCM sn: 100% RCM sp: 88.5%
Metaanalysis[93]	4163	BCC	RCM sn: 92% RCM sp: 93%
Retrospective[94]	123	BCC	RCM sn: 97.1% RCM sp: 78.95%
Cochrane meta-analysis[95]	2037	BCC (n = 464)	RCM sn: 94% RCM sp: 85%
Prospective[96]	100	BCC	RCM sn: 100% RCM sp: 38% biopsy sn: 93.94% biopsy sp: 79%
Retrospective[97]	1189	All lesion types	RCM sn: 98.2% RCM sp: 99.8%
Prospective[13]	1279	All lesion types	RCM sn: 95.3% RCM sp: 83.9%

BCC, Basal cell carcinoma; *derm*, dermosocpe; *LM*, lentigo maligna; *LMM*, lentigo maligna melanoma; *sn*, sensitivity; *sp*, specificity.

combined RCM and optical coherence tomography (OCT), which captures low-resolution images at greater depth than RCM. The study found a sensitivity of 82.6% and specificity of 93.8% for BCC diagnosis at lateral- and deep-margin evaluation after the initial biopsy procedure of BCCs.[25] Notably, RCM/OCT use demonstrated that 29% of BCCs had no residual tumor burden after the initial biopsy procedure, verified by frozen sections.[25] The ability to identify lesions with complete tumor clearance after the initial biopsy procedure may circumvent the need for further surgery or allow the clinician to pursue noninvasive treatment options.[22]

LM presentation in cosmetically sensitive areas and difficulty achieving clear surgical margins with associated significant recurrence rate after surgery, make LM a therapeutic challenge.[26-29] To that end, RCM has been used for the planning of surgical margins of LM, specifically in cases that are light-colored or amelanotic, or in recurrent cases.[29] Pellacani et al[30] evaluated the feasibility of using superficial skin cuts as RCM imaging anchors for presurgical evaluation of LM margins. They showed,

in 23 patients, that RCM correctly predicted negative margins in 91%, while clinical-dermoscopic evaluation correctly mapped margins in only 26% of lesions—verified histopathologically.[30] The utility of RCM lies in its ability to detect atypical melanocytes at LM borders that are not discernible clinically or dermoscopically.[30-32] Similarly, Yelamos et al[33] found, in 23 cases of LM and LM melanoma, concordance in margin mapping between handheld RCM (using a radial video–mosaicing technique) and staged excision. Surgical margins were a mean of 0.8 mm larger than those estimated by RCM.[33] While RCM-based margin mapping is currently limited to research studies in the United States, these results have been incorporated into evidence-based clinical practice guidelines for the management of LM in Australia.[34]

Monitoring of ablative and noninvasive therapy

RCM is also under investigation in monitoring response to noninvasive therapies.[35,36] Assessment of tumor clearance after treatment, based on clinical and dermoscopic examination, is limited because of hyperpigmentation and erythema at the treatment site.[12] Patients receiving noninvasive therapy may also be reluctant to undergo invasive biopsy procedures to ensure tumor clearance.[12] Therefore, a noninvasive means of monitoring response to treatment is warranted. Studies have explored the use of RCM after imiquimod therapy in patients with LM that were unsuitable surgical candidates.[37,38] Among LMs with histopathologically confirmed response to imiquimod therapy, RCM showed a significant decrease in the mean number of atypical melanocytes (from 121/mm^2 before to 7/mm^2 after therapy, $P < .0001$).[37] The utility of RCM in monitoring actinic keratoses after treatment has also been shown in several studies.[39-41] Before actinic keratosis treatment, RCM imaging of the epidermis showed parakeratosis and atypical honeycomb composed of atypical keratinocytes, whereas after treatment a regular honeycomb pattern was restored within the epidermis.[42]

Studies have also evaluated the role of noninvasive treatment monitoring with RCM for BCC,[43-46] particularly in disorders with the formation of numerous BCCs. Given the skin cancer burden in these patients, noninvasive therapies are ideal; however, a noninvasive means of monitoring response and ensuring tumor resolution is necessary. The utility of RCM in assessing BCC response to photodynamic therapy in patients with xeroderma pigmentosum and nevoid BCC syndrome has been demonstrated.[43] In addition, Longo et al[44] demonstrated that in 12 patients with BCCs treated with photodynamic therapy RCM detected histopathologically confirmed residual BCC that went undetected by clinical-dermoscopic examination. Similarly, evaluation of BCC response to oral hedgehog inhibitors (e.g., vismodegib) with RCM showed that pseudocyst formation ("empty tumor nests") and fibrosis were indicators of BCC regression—confirmed histopathologically.[45] Banzhaf et al[46] combined ablative fractional laser and ingenol mebutate for management of 20 BCCs and evaluated response by RCM/OCT. Repeat treatment was provided at day 29 in 25% of BCCs, in which subclinical residual tumor was detected by RCM/OCT. The RCM/OCT-estimated cure rate at day 90 was 60%, at substantial agreement with the histopathologically confirmed cure rate of 70% ($\kappa \geq 0.8$, $P < .0001$).[46] Notably, caution should be exercised to not miss deep residual disease because of RCM's depth limitation.[12,22]

Tumor recurrence surveillance

Another therapeutic challenge is the recurrence of pigmentation within a scar. The diagnostic dilemma is differentiating a recurrent nevus versus melanoma versus epidermal-basal hyperpigmentation.[47,48] If a biopsy procedure is undertaken, there is the possibility of sampling error yielding false negative results and further incurring scarring.[49] RCM can facilitate both diagnosis and locating the involved area (particularly in large lesions) to guide a high-yield biopsy specimen. The effectiveness of integrating clinical, dermoscopic, and RCM examination in determining the nature of repigmentation within scars has been demonstrated.[50] RCM alone has shown higher sensitivity than dermoscopy for evaluation of recurrent LM/LM melanoma.[51] Another study evaluated new pigmentation within or adjacent to LM/LM melanoma surgical scars with RCM and histopathology in 29 patients, demonstrating RCM sensitivity of 95.2% (95% CI 76.2-99.9%) and specificity of 77.7% (95% CI 40.0-97.2%).[49]

Applications in inflammatory disorders

Several studies have evaluated the role of RCM in inflammatory disorders.[52] Concordance between histopathologic and RCM findings have been demonstrated in psoriasis,[53] discoid lupus erythematosus,[54] contact dermatitis,[55] and lichen planus.[56] RCM can also aid in the classification of inflammatory patterns that can be recognized from assessment of the epidermis and superficial dermis—psoriasiform dermatitis, interface dermatitis, and spongiotic dermatitis.[52] However, RCM use in inflammatory disorders is limited by depth

Table IV. Limitations and artifacts of reflectance confocal microscopy imaging

Limitations	Examples
Related to its optical, mechanical, and software properties	Illumination artifacts[98]: AIC unit should adjust laser power intensity to attain uniform level of illumination across images. Failure of AIC to function properly results in oversaturated or underilluminated areas. This tends to occur at areas with sharp transition in brightness (e.g., near hair follicles). Mosaic stitching artifacts: The RCM acquires adjacent optical sections and the software stiches them into mosaic images. The device mechanically moves the skin relative to the laser beam during mosaic acquisition. Failure of this process may cause repeat appearance of the same structure in adjacent tiles of the mosaic or in uneven transition between tiles (giving a "checkerboard" appearance to the mosaic; Figure 7). Depth: Maximum imaging depth is about 250 μm. With increasing depth, loss of resolution occurs. Field of view: The handheld device has a field-of-view limited to 0.75 $\times$ 0.75 mm^2. This narrow field of view does not allow low-magnification assessment of pattern symmetry or comparison of overall RCM pattern to the dermoscopic pattern, which are often needed in differentiating atypical nevi from melanoma.
Related to patient and lesion factors	Motion artifact[98]: Imaging disruption resulting from patient movement. For example, breathing may cause uneven imaging depth during capture of mosaic images. Excess backscatter[98-100]: Hyperkeratotic and heavily pigmented lesions can cause excess superficial backscattering of light causing oversaturation of superficial images and hindering visualization of deeper structures. In addition, air bubbles and external reflective particles (e.g., sunscreens) may strongly backscatter RCM light and obscure underlying structures. Anatomic site—related limitations[101]: • In palmoplantar skin, the DEJ may difficult to visualize because of the thickness of the epidermis • On a hairy scalp, the hairs strongly back-scatter light and interfere with imaging • On narrow or concave anatomic sites, RCM requires full contact with the skin. The wide-probe RCM requires a flat larger surface. The handheld RCM has a smaller probe that can access most anatomic sites but may be restricted in regions like interdigital spaces or inner surface of the auricles Dendritic cells[59]: Langerhans cells may be difficult to distinguish from melanocytes under RCM. In inflamed pigmented lesions (e.g., lichen planus like keratosis), Langerhans cells may become conspicuous, and may be erroneously interpreted as being dendritic melanocytes in Pagetoid spread, leading to false-positive diagnoses of melanoma.

AIC, Automatic intensity control; *DEJ*, dermoepidermal junction; *RCM*, reflectance confocal microscopy.

visualization restrictions and by difficulty in differentiating between various types of inflammatory cells.

Limitations of RCM imaging

Awareness of the limitations and pitfalls of RCM is paramount. To avoid false negative RCM diagnosis, users should be cautious with: (1) nodular lesions because of limited RCM depth penetration—this is akin to a missed diagnosis of nodular melanoma because of a superficial shave biopsy specimen; (2) lesions with substantial hyperkeratosis, crusting or ulceration—as the highly reflective surface may reduce imaging quality; and (3) lesions with discrepancy between the clinical-dermoscopic and RCM findings.[57,58] Table IV provides a summary of key RCM limitations.

PRACTICAL CONSIDERATIONS

Billing

On January 1, 2016, category I *Current Procedural Terminology* reimbursement codes were established for the use of the wide-probe RCM (96931-96936) for image acquisition and image interpretation. The first 3 codes are used for imaging a single lesion. Code 96931 applies if the same physician's office staff acquired the images and then that physician interpreted images and produced a report.[59] Codes 96932 and 96933 apply if 2 separate physicians were involved in ordering image acquisition and performing interpretation. For each additional lesion, there are 3 corresponding codes that correlate with the aforementioned (96934, 96935, and 96936).[59] For the first lesion, the average relative value unit granted is 3.57 for image acquisition and 1.30 for interpretation, which is comparable to a biopsy procedure and

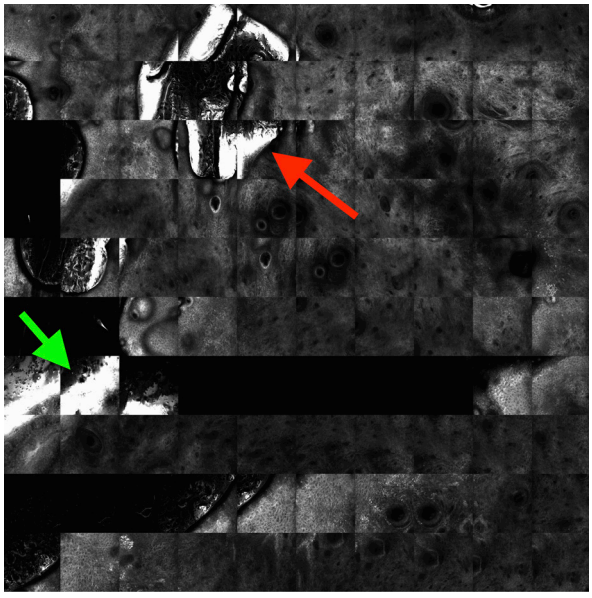

Fig 7. Reflectance confocal microscopy mosaic depicting an air bubble appearing highly refractile (red arrow), mosaic stitching artifact, and illumination artifact at areas of sharp transition (green arrow).

histopathologic interpretation. Further information is available in the referenced video.[60]

Logistics

Although the cost of the device is high, the presence of reimbursement codes and options to lease afford prospects for practices to add RCM to their toolbox. Training is required for both the individual acquiring images (usually a technician or nurse), as well as those interpreting the images and creating the report. Training for initial image acquisition takes about 1 month, with a subsequent learning curve of 3 additional months. Training for image interpretation is estimated to take 4 to 6 months.[12,61] As most US training programs do not teach RCM at present, learning is self-tailored and involves RCM courses taught by experts,[62-64] online educational resources,[65-69] and textbooks.[70-72] It is helpful to seek occasional guidance from an experienced RCM user during the first 6 months.[73] We recommend dedicating long-term staff for image acquisition because of the relatively lengthy learning curve.

Image acquisition of a single lesion typically ranges in time, but approximates the time needed to set up, explain, and perform a biopsy procedure; image acquisition with the handheld is significantly faster. The recommended image set for the wide-probe RCM is a minimum of 3 mosaic images at the suprabasal epidermis, basal epidermis/DEJ, and superficial dermis. For both wide-probe and handheld RCMs, optical sections or image stacks are obtained at foci that show suspicious RCM findings.

When use of RCM is deemed appropriate, that patient can have image acquisition during the same appointment or, if not possible, at a scheduled return visit soon thereafter. The ordering dermatologist specifies the lesion of interest and the staff acquires the RCM images. The images can be read by the bedside or forwarded to the physician providing interpretation. The RCM is signed out like a dermatopathology report and sent to the ordering dermatologist.

CONCLUSION

Use of RCM is increasing to help diagnose equivocal neoplasms and reduce unnecessary biopsy procedures in patients with benign neoplasms. The role of RCM is evolving in presurgical margin mapping, recognition of tumor recurrence, monitoring of noninvasive therapies, and inflammatory skin disease.

REFERENCES

1. Kittler H, Pehamberger H, Wolff K, Binder M. Diagnostic accuracy of dermoscopy. *Lancet Oncol*. 2002;3:159-165.
2. Vestergaard ME, Macaskill P, Holt PE, Menzies SW. Dermoscopy compared with naked eye examination for the diagnosis of primary melanoma: a meta-analysis of studies performed in a clinical setting. *Br J Dermatol*. 2008;159:669-676.
3. Minsky M. Microscopy Apparatus. US Patent 3,013,467. 1961.
4. Ahlgrimm-Siess V, Langley RGB, Hofmann-Wellenhof R. Solar lentigo, seborrheic keratosis and lichen planus-like keratosis. In: Hofmann-Wellenhof R, Pellacani G, Malvehy J, Soyer HP, eds. *Reflectance Confocal Microscopy for Skin Diseases*. 1st ed. New York: Springer; 2012:259-273.
5. Calzavara-Pinton P, Longo C, Venturini M, Sala R, Pellacani G. Reflectance confocal microscopy for in vivo skin imaging. *Photochem Photobiol*. 2008;84:1421-1430.
6. Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. *J Invest Dermatol*. 1995;104:946-952.
7. Scope A, Benvenuto-Andrade C, Agero ALC, et al. In vivo reflectance confocal microscopy imaging of melanocytic skin lesions: consensus terminology glossary and illustrative images. *J Am Acad Dermatol*. 2007;57:644-658.
8. Huzaira M, Rius F, Rajadhyaksha M, Anderson RR, González S. Topographic variations in normal skin, as viewed by in vivo reflectance confocal microscopy. *J Invest Dermatol*. 2001;116:846-852.
9. Rajadhyaksha M, González S, Zavislan JM, Anderson RR, Webb RH. In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. *J Invest Dermatol*. 1999;113:293-303.
10. Busam KJ, Charles C, Lee G, Halpern AC. Morphologic features of melanocytes, pigmented keratinocytes, and melanophages by in vivo confocal scanning laser microscopy. *Mod Pathol*. 2001;14:862-868.

11. Busam KJ, Marghoob AA, Halpern A. Melanoma diagnosis by confocal microscopy: promise and pitfalls. *J Invest Dermatol*. 2005;125:vii.
12. Levine A, Markowitz O. Introduction to reflectance confocal microscopy and its use in clinical practice. *JAAD Case Rep*. 2018;4:1014-1023.
13. Borsari S, Pampena R, Lallas A, et al. Clinical indications for use of reflectance confocal microscopy for skin cancer diagnosis. *JAMA Dermatol*. 2016;152:1093-1098.
14. Stevenson A, Mickan S, Mallett S, Ayya M. Systematic review of diagnostic accuracy of reflectance confocal microscopy for melanoma diagnosis in patients with clinically equivocal skin lesions. *Dermatol Pract Concept*. 2013;3:19-27.
15. Kadouch DJ, Schram ME, Leeflang MM, Limpens J, Spuls PI, De Rie MA. In vivo confocal microscopy of basal cell carcinoma: a systematic review of diagnostic accuracy. *J Eur Acad Dermatol Venereol*. 2015;29:1890-1897.
16. Nguyen KP, Peppelman M, Hoogedoorn L, Van Erp PEJ, Gerritsen MJP. The current role of in vivo reflectance confocal microscopy within the continuum of actinic keratosis and squamous cell carcinoma: a systematic review. *Eur J Dermatol*. 2016;26:549-565.
17. Xiong YD, Ma S, Li X, Zhong X, Duan C, Chen Q. A meta-analysis of reflectance confocal microscopy for the diagnosis of malignant skin tumours. *J Eur Acad Dermatol Venereol*. 2016;30:1295-1302.
18. Yélamos O, Manubens E, Jain M, et al. Improvement of diagnostic confidence and management of equivocal skin lesions by integration of reflectance confocal microscopy in daily practice: prospective study in two referral skin cancer centers [e-pub ahead of print]. *J Am Acad Dermatol*. https://doi.org/10.1016/j.jaad.2019.05.101. Accessed August 24, 2020.
19. Pellacani G, Pepe P, Casari A, Longo C. Reflectance confocal microscopy as a second-level examination in skin oncology improves diagnostic accuracy and saves unnecessary excisions: a longitudinal prospective study. *Br J Dermatol*. 2014;171:1044-1051.
20. Alarcon I, Carrera C, Palou J, Alos L, Malvehy J, Puig S. Impact of in vivo reflectance confocal microscopy on the number needed to treat melanoma in doubtful lesions. *Br J Dermatol*. 2014;170:802-808.
21. Stanganelli I, Longo C, Mazzoni L, et al. Integration of reflectance confocal microscopy in sequential dermoscopy follow-up improves melanoma detection accuracy. *Br J Dermatol*. 2015;172:365-371.
22. Holmkvist KA, Rogers GS, Dahl PR. Incidence of residual basal cell carcinoma in patients who appear tumor free after biopsy. *J Am Acad Dermatol*. 1999;41:600-605.
23. Navarrete-Dechent C, Cordova M, Aleissa S, et al. Reflectance confocal microscopy confirms residual basal cell carcinoma on clinically negative biopsy sites before Mohs micrographic surgery: a prospective study. *J Am Acad Dermatol*. 2019;81:417-426.
24. Sasor SE, Nosrati NN, Katona T, et al. Predicting the presence of nonmelanoma skin cancers after biopsy: a method to reduce unnecessary surgical procedures. *JAMA Surg*. 2016;15:289-290.
25. Aleissa S, Navarrete-Dechent C, Cordova M, et al. Presurgical evaluation of basal cell carcinoma using combined reflectance confocal microscopy-optical coherence tomography: a prospective study. *J Am Acad Dermatol*. 2019;82:962-968.
26. King R. Lentiginous melanoma. *Arch Pathol Lab Med*. 2011;31:281-288.
27. McLeod M, Choudhary S, Giannakakis G, Nouri K. Surgical treatments for lentigo maligna: a review. *Dermatol Surg*. 2011;37:1210-1228.
28. Hazan C, Dusza SW, Delgado R, Busam KJ, Halpern AC, Nehal KS. Staged excision for lentigo maligna and lentigo maligna melanoma: a retrospective analysis of 117 cases. *J Am Acad Dermatol*. 2008;58:142-148.
29. Guitera P, Moloney FJ, Menzies SW, et al. Improving management and patient care in lentigo maligna by mapping with in vivo confocal microscopy. *JAMA Dermatol*. 2013;149:692-698.
30. Pellacani G, De Carvalho N, Ciardo S, et al. The smart approach: feasibility of lentigo maligna superficial margin assessment with hand-held reflectance confocal microscopy technology. *J Eur Acad Dermatol Venereol*. 2018;32:1687-1694.
31. Guitera P, Pellacani G, Crotty KA, et al. The impact of in vivo reflectance confocal microscopy on the diagnostic accuracy of lentigo maligna and equivocal pigmented and nonpigmented macules of the face. *J Invest Dermatol*. 2010;130:2080-2091.
32. De Carvalho N, Farnetani F, Ciardo S, et al. Reflectance confocal microscopy correlates of dermoscopic patterns of facial lesions help to discriminate lentigo maligna from pigmented nonmelanocytic macules. *Br J Dermatol*. 2015;173:128-133.
33. Yélamos O, Cordova M, Blank N, et al. Correlation of handheld reflectance confocal microscopy with radial video mosaicing for margin mapping of lentigo maligna and lentigo maligna melanoma. *JAMA Dermatol*. 2017;153:1278-1284.
34. Robinson M, Primiero C, Guitera P, et al. Evidence-based clinical practice guidelines for the management of patients with lentigo maligna. *Dermatology*. 2020;236:111-116.
35. González S, Swindells K, Rajadhyaksha M, Torres A. Changing paradigms in dermatology: confocal microscopy in clinical and surgical dermatology. *Clin Dermatol*. 2003;21:359-369.
36. Guilera JM, Barreiro Capurro A, Carrera Alvárez C, Puig Sardá S. The role of reflectance confocal microscopy in clinical trials for tumor monitoring. *Dermatol Clin*. 2016;34:519-526.
37. Brand FL, Seyed Jafari SM, Hunger RE. Confocal microscopy and lentigo maligna: an in vivo pilot study for the assessment of response to imiquimod therapy. *Dermatology*. 2019;235:150-155.
38. Alarcon I, Carrera C, Alos L, Palou J, Malvehy J, Puig S. In vivo reflectance confocal microscopy to monitor the response of lentigo maligna to imiquimod. *J Am Acad Dermatol*. 2014;71:49-55.
39. Ulrich M, Krueger-Corcoran D, Roewert-Huber J, Sterry W, Stockfleth E, Astner S. Reflectance confocal microscopy for noninvasive monitoring of therapy and detection of subclinical actinic keratoses. *Dermatology*. 2010;220:15-24.
40. Malvehy J, Alarcon I, Montoya J, Rodríguez-Azeredo R, Puig S. Treatment monitoring of 0.5% 5-fluorouracil and 10% salicylic acid in clinical and subclinical actinic keratoses with the combination of optical coherence tomography and reflectance confocal microscopy. *J Eur Acad Dermatol Venereol*. 2016;30:258-265.
41. Maier T, Cekovic D, Ruzicka T, Sattler EC, Berking C. Treatment monitoring of topical ingenol mebutate in actinic keratoses with the combination of optical coherence tomography and reflectance confocal microscopy: a case series. *Br J Dermatol*. 2015;172:816-818.
42. Longo C, Borsari S, Benati E, Moscarella E, Alfano R, Argenziano G. Dermoscopy and reflectance confocal microscopy for monitoring the treatment of actinic keratosis with

ingenol mebutate gel: report of two cases. *Dermatol Ther (Heidelb)*. 2016;6:81-87.
43. Segura S, Puig S, Carrera C, Lecha M, Borges V, Malvehy J. Non-invasive management of non-melanoma skin cancer in patients with cancer predisposition genodermatosis: a role for confocal microscopy and photodynamic therapy. *J Eur Acad Dermatol Venereol*. 2011;25:819-827.
44. Longo C, Casari A, Pepe P, et al. Confocal microscopy insights into the treatment and cellular immune response of basal cell carcinoma to photodynamic therapy. *Dermatology*. 2013;225:264-270.
45. Maier T, Kulichova D, Ruzicka T, Berking C. Noninvasive monitoring of basal cell carcinomas treated with systemic hedgehog inhibitors: pseudocysts as a sign of tumor regression. *J Am Acad Dermatol*. 2014;71:725-730.
46. Banzhaf CA, Phothong W, Suku MH, et al. Basal cell carcinoma treated with combined ablative fractional laser and ingenol mebutate – an exploratory study monitored by optical coherence tomography and reflectance confocal microscopy. *J Eur Acad Dermatol Venereol*. 2020;34:502-509.
47. Connolly KL, Nijhawan RI, Dusza SW, Busam KJ, Nehal KS. Time to local recurrence of lentigo maligna: implications for future studies. *J Am Acad Dermatol*. 2016;74:1247-1248.
48. Kunishige JH, Brodland DG, Zitelli JA. Surgical margins for melanoma in situ. *J Am Acad Dermatol*. 2012;66:438-444.
49. Navarrete-Dechent C, Cordova M, Liopyris K, et al. Reflectance confocal microscopy and dermoscopy aid in evaluating repigmentation within or adjacent to lentigo maligna melanoma surgical scars. *J Eur Acad Dermatol Venereol*. 2020;34:74-81.
50. Longo C, Moscarella E, Pepe P, et al. Confocal microscopy of recurrent naevi and recurrent melanomas: a retrospective morphological study. *Br J Dermatol*. 2011;165:61-68.
51. Cinotti E, Labeille B, Debarbieux S, et al. Dermoscopy vs. reflectance confocal microscopy for the diagnosis of lentigo maligna. *J Eur Acad Dermatol Venereol*. 2018;32:1284-1291.
52. Ardigo M, Agozzino M, Franceschini C, Lacarrubba F. Reflectance confocal microscopy algorithms for inflammatory and hair diseases. *Dermatol Clin*. 2016;34:487-496.
53. Ardigo M, Cota C, Berardesca E, González S. Concordance between in vivo reflectance confocal microscopy and histology in the evaluation of plaque psoriasis. *J Eur Acad Dermatol Venereol*. 2009;23:660-667.
54. Ardigò M, Maliszewski I, Cota C, et al. Preliminary evaluation of in vivo reflectance confocal microscopy features of discoid lupus erythematosus. *Br J Dermatol*. 2007;156:1196-1203.
55. Astner S, González S, Gonzalez E. Noninvasive evaluation of allergic and irritant contact dermatitis by in vivo reflectance confocal microscopy. *Dermatitis*. 2006;17:182-191.
56. Moscarella E, González S, Agozzino M, et al. Pilot study on reflectance confocal microscopy imaging of lichen planus: a real-time, non-invasive aid for clinical diagnosis. *J Eur Acad Dermatol Venereol*. 2012;26:1258-1265.
57. Scope A, Marchetti MA. An evolving approach to the detection of melanoma and other skin cancers using in vivo reflectance confocal microscopy. *JAMA Dermatol*. 2016;152:1085-1087.
58. Coco V, Farnetani F, Cesinaro AM, et al. False-negative cases on confocal microscopy examination: a retrospective evaluation and critical reappraisal. *Dermatology*. 2016;232:189-197.
59. Rajadhyaksha M, Marghoob A, Rossi A, Halpern AC, Nehal KS. Reflectance confocal microscopy of skin in vivo: from bench to bedside. *Lasers Surg Med*. 2017;49:7-19.
60. Siegel DM. Reflectance confocal microscopy coding. Available at: https://caliberid.com/rcm2018.html. Accessed April 12, 2020.
61. Edwards S, Mavranezouli I, Osei-Assibey G, Marceniuk G, Wakefield V, Karner C. Vivascope 1500 and 3000 systems for detecting and monitoring skin lesions: a systematic review and economic evaluation. *Health Technol Assess*. 2016;20:1-260.
62. Memorial Sloan Kettering website. 2nd annual basic course in reflectance confocal microscopy: noninvasive diagnosis of skin cancer. Available at: https://mskcc.cloud-cme.com/default.aspx?P=5&EID=13989. Accessed April 12, 2020.
63. Noninvasive Diagnostic Innovations in Skin (NIDIskin) website. NIDIskin fall symposium: confocal microscopy reader's course. Available at: https://www.nidiskin.com/nidiskin-fall-symposium/. Accessed April 12, 2020.
64. 2nd World Congress on Confocal Microscopy. Available at: 2ndWorldCongress.eventbrite.com. Accessed April 12, 2020.
65. Confocal Microscopy 101 website. Available at: https://confocal101.com. Accessed April 12, 2020.
66. Confocaltraining.com website. Avaiable at: https://www.confocaltraining.com/tutorial. Accessed April 12, 2020.
67. Rao BK. Confocal microscopy of non-melanocytic skin lesions: squamous cell, basal cell carcinoma, other common neoplasms. Available at: https://documents.cap.org/documents/confocal-microscopy-lesions.pdf. Accessed April 12, 2020.
68. VivaScope website. Online booklet, melanoma and pigmented lesion research. Available at: http://www.vivascope-pub.com/medical/in-vivo/melanoma-pigmented-lesion-research.html. Accessed April 12, 2020.
69. International Confocal Group website. Available at: https://www.confocal-icwg.com/. Accessed April 12, 2020.
70. Hofmann-Wellenhof R, Pellacani G, Malvehy J, Soyer HP. *Reflectance Confocal Microscopy*. 1st ed. Heidelberg, Germany: Springer; 2012.
71. Gonzalez S, Gill M, Halpern AC. *Reflectance Confocal Microscopy of Cutaneous Tumors: An Atlas with Clinical, Dermoscopic and Histological Correlations*. 1st ed. Boca Raton, FL: CRC Press; 2008.
72. Gonzalez S. *Reflectance Confocal Microscopy in Dermatology: Fundamentals and Clinical Applications*. 1st ed. Madrid, Spain: Aulamedica; 2012.
73. Rao BK, Mateus R, Wassef C, Pellacani G. In vivo confocal microscopy in clinical practice: comparison of bedside diagnostic accuracy of a trained physician and distant diagnosis of an expert reader. *J Am Acad Dermatol*. 2013;69:e295-e300.
74. Que SKT, Fraga-Braghiroli N, Grant-Kels JM, Rabinovitz HS, Oliviero M, Scope A. Through the looking glass: basics and principles of reflectance confocal microscopy. *J Am Acad Dermatol*. 2015;73:276-284.
75. Huang X, Protheroe MD, Al-Jumaily AM, Paul SP, Chalmers AN. Review of human hair optical properties in possible relation to melanoma development. *J Biomed Opt*. 2018;23:1-9.
76. Arifler D, Pavlova I, Gillenwater A, Richards-Kortum R. Light scattering from collagen fiber networks: micro-optical properties of normal and neoplastic stroma. *Biophys J*. 2007;92:3260-3274.
77. Gajinov Z, Matić M, Prćić S, Đuran V. Optical properties of the human skin. *Serbian J Dermatol Venerol*. 2013;2:131-136.
78. Ding H, Lu JQ, Wooden WA, Kragel PJ, Hu XH. Refractive indices of human skin tissues at eight wavelengths and estimated dispersion relations between 300 and 1600 nm. *Phys Med Biol*. 2006;51:1479-1489.

79. Kinnunen M, Kauppila A, Karmenyan A, Myllylä R. Effect of the size and shape of a red blood cell on elastic light scattering properties at the single-cell level. *Biomed Opt Express*. 2011;2:1803-1814.
80. Maltsev VP, Hoekstra AG, Yurkin MA. Optics of white blood cells: optical models, simulations, and experiments. In: *Advanced Optical Flow Cytometry: Methods and Disease Diagnoses*. Hoboken, NJ: Wiley-WCH; 2011:63-93.
81. Liu S, Deng Z, Li J, Wang J, Huang N. Measurement of the refractive index of whole blood and its components for a continuous spectral region. *J Biomed Opt*. 2019;24:1-5.
82. Dunn A, Richards-Kortum R. Three-dimensional computation of light scatteing from cells. *IEEE J Sel Top Quantum Electron*. 1996;2:898-905.
83. Shahriari N, Grant-Kels JM, Rabinovitz H, Oliviero M, Scope A. In vivo reflectance confocal microscopy image interpretation for the dermatopathologist. *J Cutan Pathol*. 2018;45:187-197.
84. Scope A, Guitera P, Pellacani G. RCM diagnosis of melanocytic neoplasms: terminology, algorithms and their accuracy, and clinical integration. In: González S, ed. *Reflectance Confocal Microscopy of Cutaneous Tumors*. 2nd ed. Boca Raton, FL: CRC Press; 2017.
85. Pellacani G, Scope A, Gonzalez S, et al. Reflectance confocal microscopy made easy: the 4 must-know key features for the diagnosis of melanoma and nonmelanoma skin cancers. *J Am Acad Dermatol*. 2019;81:520-526.
86. Hao T, Meng XF, Li CX. A meta-analysis comparing confocal microscopy and dermoscopy in diagnostic accuracy of lentigo maligna [e-pub ahead of print]. *Ski Res Technol*. https://doi.org/10.1111/srt.12821. Accessed August 24, 2020.
87. Lan J, Wen J, Cao S, et al. The diagnostic accuracy of dermoscopy and reflectance confocal microscopy for amelanotic/hypomelanotic melanoma: a systematic review and meta-analysis. *Br J Dermatol*. 2020;183:210-219.
88. Guitera P, Pellacani G, Longo C, Seidenari S, Avramidis M, Menzies SW. In vivo reflectance confocal microscopy enhances secondary evaluation of melanocytic lesions. *J Invest Dermatol*. 2009;129:131-138.
89. Gerger A, Koller S, Kern T, et al. Diagnostic applicability of in vivo confocal laser scanning microscopy in melanocytic skin tumors. *J Invest Dermatol*. 2005;124:493-498.
90. Dinnes J, Deeks JJ, Saleh D, et al. Reflectance confocal microscopy for diagnosing cutaneous melanoma in adults. *Cochrane Database Syst Rev*. 2018;12:CD013190.
91. Lovatto L, Carrera C, Salerni G, Alõs L, Malvehy J, Puig S. In vivo reflectance confocal microscopy of equivocal melanocytic lesions detected by digital dermoscopy follow-up. *J Eur Acad Dermatol Venereol*. 2015;29:1918-1925.
92. Guitera P, Menzies SW, Longo C, Cesinaro AM, Scolyer RA, Pellacani G. In vivo confocal microscopy for diagnosis of melanoma and basal cell carcinoma using a two-step method: analysis of 710 consecutive clinically equivocal cases. *J Invest Dermatol*. 2012;132:2386-2394.
93. Lupu M, Popa IM, Voiculescu VM, Caruntu A, Caruntu C. A systematic review and meta-analysis of the accuracy of in vivoreflectance confocal microscopy for the diagnosis of primary basal cell carcinoma. *J Clin Med*. 2019;8:E1462.
94. Lupu M, Popa I, Voiculescu V, et al. A retrospective study of the diagnostic accuracy of in vivo reflectance confocal microscopy for basal cell carcinoma diagnosis and subtyping. *J Clin Med*. 2019;8:E499.
95. Dinnes J, Deeks JJ, Chuchu N, et al. Reflectance confocal microscopy for diagnosing keratinocyte skin cancers in adults. *Cochrane Database Syst Rev*. 2018;12:CD013191.
96. Kadouch DJ, Leeflang MM, Elshot YS, et al. Diagnostic accuracy of confocal microscopy imaging vs. punch biopsy for diagnosing and subtyping basal cell carcinoma. *J Eur Acad Dermatol Venereol*. 2017;31:1641-1648.
97. Rao BK, John AM, Francisco G, Haroon A. Diagnostic accuracy of reflectance confocal microscopy for diagnosis of skin lesions: an update. *Arch Pathol Lab Med*. 2019;143:326-329.
98. Gill M, Alessi-Fox C, Kose K. Artifacts and landmarks: pearls and pitfalls for in vivo reflectance confocal microscopy of the skin using the tissue-coupled device. *Dermatol Online J*. 2019;25:0-13.
99. Ulrich M, Zalaudek I, Welzel J. Shining into the white: the spectrum of epithelial tumors from actinic keratosis to squamous cell carcinoma. *Dermatol Clin*. 2016;34:459-467.
100. Longo C, Farnetani F, Ciardo S, et al. Is confocal microscopy a valuable tool in diagnosing nodular lesions? A study of 140 cases. *Br J Dermatol*. 2013;169:58-67.
101. Serban ED, Farnetani F, Pellacani G, Constantin MM. Role of in vivo reflectance confocal microscopy in the analysis of melanocytic lesions. *Acta Dermatovenerol Croat*. 2018;26:64-67.