ORIGINAL ARTICLE

International expert recommendations on image acquisition for in vivo reflectance confocal microscopy of cutaneous tumors

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Background: No international recommendations exist for a minimum imaging requirement per lesion using reflectance confocal microscopy (RCM). This may be beneficial given the increasing use of remote RCM interpretation internationally.

Objective: To develop international expert recommendations for image acquisition using tissue-coupled RCM for diagnosis of cutaneous tumors.

Methods: Using a modified Delphi approach, a core group developed the scope and drafted initial recommendations before circulation to a larger group, the Cutaneous Imaging Expert Resource Group of the American Academy of Dermatology. Each review round consisted of a period of open comment, followed by revisions.

Results: The recommendations were developed after 5 alternating rounds of review among the core group and the Cutaneous Imaging Expert Resource Group. These were divided into subsections of imaging personnel, recommended lesion criteria, clinical and lesion information to be provided, lesion preparation, image acquisition, mosaic cube settings, and additional captures based on lesion characteristics and suspected diagnosis.

Limitations: The current recommendations are limited to tissue-coupled RCM for diagnosis of cutaneous tumors. It is one component of the larger picture of quality assurance and will require ongoing review.

Conclusions: These recommendations serve as a resource to facilitate quality assurance, economical use of time, accurate diagnosis, and international collaboration. (J Am Acad Dermatol https://doi.org/10.1016/j.jaad.2023.09.086.)

Key words: cutaneous tumors; imaging; recommendations; reflectance confocal microscopy; technology; teledermatology.

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BACKGROUND

The use of reflectance confocal microscopy (RCM) has increased dramatically in the last decade, aiding in the diagnosis of cutaneous malignancies.[1] In 2016, the US Centers for Medicare and Medicaid Services (CMS) granted Current Procedural Terminology physician reimbursement codes, subdivided into image acquisition only, interpretation and report only, or both, for investigated lesions.[2] Remote interpretation using a store-and-forward method is expected to grow with increased use of telemedicine. Trials using this method are ongoing in Australia and Chile, whereas the United States has specific reimbursement codes for RCM, including that for remote interpretation.

CAPSULE SUMMARY

- These international recommendations developed by experts provide guidance on optimal image capture for in-vivo reflectance confocal microscopy of cutaneous tumors using the tissue-coupled device.
- They have been developed to help assure quality and facilitate remote diagnosis.

Existing protocols have been described in the literature but not standardized internationally.[3-8] The current US CMS reimbursement model applies only to tissue-couple devices (such as the VivaScope 1500, Caliber ID) that capture wide-field mosaics with dermatoscopy image correlation. Handheld devices that only capture stacks and do not capture mosaics currently do not have CMS reimbursement codes and are not covered in this article. Image types captured by existing commercial devices have been described elsewhere.[2,3] To be reimbursed, the US CMS code requires a minimum standard of 4 mosaic images with at least one each captured in the suprabasal epidermis, basal epidermis/dermoepidermal junction, and papillary dermis; stacks at areas of interest are optional.[2,9] However, this minimum image set does not define the specific parameters that may be required to capture all information on diagnostic or therapeutic interest for every lesion type. Although device manufacturers train technicians to a level of basic proficiency, the expertise of the imaging technicians within the existing literature may not be reflective of that of the average user in a real-life clinical setting. Therefore, this "one size fits all" approach may be less efficient in the setting of asynchronous remote interpretation.

RCM image acquisition recommendations are needed to ensure a quality image set, taking into consideration economical use of time for the patient, the technician acquiring the images, and the physician interpreting the images; facilitate accurate diagnosis by accounting for lesions' specific requirements regardless of the technician's prior knowledge or lesion location; and encourage and enable collaborations and/or consultation across multiple sites and/or countries. Similar recommendations have been created for clinical and dermatoscopic imaging of skin lesions in an effort to standardize the techniques used.[10,11]

Aims and scope

The aim of this study was to develop international consensus recommendations to define a recommended minimum image set for in vivo tissue-coupled RCM evaluation of skin tumors. To achieve this goal, a detailed protocol including optimal type and number of RCM images necessary and sufficient to accurately diagnose any tumor was created by a panel of experts. These recommendations are intended for use by imaging technicians and supervising physicians billing for imaging. Supervising physicians should know and understand these protocols so they can provide technicians with the information needed to optimize the imaging procedure, as is done with other imaging modalities. The recommendations reported herein are specific to tissue-coupled (wide-probe) RCM when used to evaluate cutaneous lesions suspicious for malignancy. Protocols for imaging inflammatory, mucosal, or vascular lesions are not covered by these recommendations. These recommendations are neither intended to cover basic steps of device setup nor intended to overcome inherent limitations of RCM.

METHODS AND CONSENSUS PROCESS

A core group developed the scope and drafted initial recommendations before circulation to a wider group of international experts. The core group consisted of 8 members: 4 dermatologists with 18 (G.P.), 25 (S.G.), 17 (P.G.), and 12 years (R.J.S.) of experience with RCM; 1 dermatologist/dermatopathologist with 12 years of RCM experience as a clinician and interpreter (J.G.-K.); 1 pathologist/dermatopathologist with 17 years of RCM experience largely as a remote interpreter (M.G.); 1 general physician/Masters student with 1 year of RCM experience (G.H.); and an engineer scientist with 25 years of RCM experience (C.A.-F.) at the time of data collection.

Abbreviations used:

CIERG: Cutaneous Imaging Expert Resource Group
CMS: Centers for Medicare and Medicaid Services
RCM: reflectance confocal microscopy

The recommendations were developed using a modified Delphi approach. The initial draft was developed after a literature review of existing imaging protocols in PubMed, MEDLINE, and Embase. Key words used were "reflectance confocal microscopy" and ("acquisition" or "remote") and ("guideline" or "guidelines" or "recommendation"). The initial draft based on literature review and personal experience was developed by 5 of the 8 core group members and then presented to a larger group of confocal users, the Cutaneous Imaging Expert Resource Group (CIERG) of the American Academy of Dermatology, for feedback in August 2021. Next, all 8 core group members performed 3 consensus rounds to develop the second version, which was again presented for feedback to CIERG in February 2022. The 8 core group members considered the feedback and developed the final version of the recommendations.

To enable broad participation, we provided synchronous and asynchronous opportunities, including live presentation with open discussion at CIERG meetings and, in parallel, electronic distribution of the presentation and recommendations with a 4-week open comment period via email, to all CIERG members (73 in August 2021 and 76 in February 2022). Several reminders were sent prior to each presentation and during the subsequent open comment period.

RESULTS

The final recommendations, including the minimum basic image set and automated cube settings, are presented in Table I. Table II[12-14] describes recommendations regarding session maps (a dermatoscopic or clinical image of the lesion indicating location of RCM images to orientate the reader) and procedures to overcome site- and lesion-related imaging challenges. Table III[6,7,13-17] lists recommendations for additional captures based on suspected diagnoses and RCM features. Consensus could not be achieved for 4 recommendations (Tables I, part A 1, 2.2, and 3.1.2, and III, part B). Of note, the specific features in Table III, part B were not the source of disagreement, but rather whether they are relevant to include as an average technician is not expected to recognize these features. Further recommendations covering imaging personnel, lesion criteria and preparation, and collection of clinical information are provided in Supplementary Tables I and II (available via Mendeley at https://data.mendeley.com/datasets/kgv9w26ckg/1). Specific recommendations and quality control steps that complement prior publications are illustrated in detail in Supplementary Figures 1 to 3 (available via Mendeley at https://data.mendeley.com/datasets/kgv9w26ckg/1).[12,13,18]

DISCUSSION

These recommendations have incorporated strategies that can be implemented to overcome variations in imaging technician training, lesion specific, and site-specific challenges while maximizing the likelihood of capturing a diagnostic image set. Economical use of imaging and reading time is integral to achieve a quality representative image set. Capturing innumerable mosaics and stacks to cover all lesion types, technical challenges, and differences reported in prior literature is neither feasible nor practical from an image capture, interpretation, transfer, or storage perspective. Although alternatives, such as live-interactive tele-RCM, have been proposed, not all centers can access an RCM expert in real time.[19]

There were major areas of disagreement within the core group. Some were resolved, but consensus could not be reached for 5 recommendations. The 2 themes of disagreement were expectations in training/experience of the technician and referring physician and the balance between concise, easily achieved recommendations and optimizing diagnostic quality. For example, the number of stacks captured in the basic set could not be agreed upon (Table I, part A 1), specifically whether they should be included under the basic set or later under specific circumstances. Some believed only 1 was enough; others recommended a minimum of 4 to increase the likelihood of capturing diagnostic structures and enable inclusion of control surrounding skin. A minimum range of 1 to 3 was chosen to balance both views. The fifth mosaic (Table I, part A 2.2) could not be agreed on because experts who solely performed remote reading believed that it was helpful as quality control and to guide management. The group could not agree on recommendations requiring technicians to identify and target dermatoscopic and/or RCM features (Tables I, part A 3.1.2, and III, part B). There was agreement that these features would be ideal to target, but some experts argued that technicians cannot be expected to achieve the skill level required to identify these structures. Further, they surmised that those who

Table I. Recommended basic image set for reflectance confocal microscopy evaluation of cutaneous tumors

Part A. Basic image set defined

1. A "basic image set," including a minimum of 4 standard mosaics and at least 1-3* standard stacks, should be acquired per lesion regardless of suspected diagnosis.
2. Mosaics (captured manually or using automated cube—see Table I, part B):
   - 2.1. Standard mosaics (total 4) shall:
     - 2.1.1 Be centered on the lesion
     - 2.1.2 Include a rim (1 mm) of nonlesional skin, if possible
     - 2.1.3 Capture the following anatomical levels:
       - Upper epidermis (granular/spinous)
       - Lower epidermis (basal layer)
       - Dermoepidermal junction
       - Papillary dermis
   - 2.2. Recommended additional deeper mosaic* to determine representativeness of imaging and lesion depth: capture just below lesion or within the lesion where loss of resolution begins to occur.
3. Stack captures (must be captured manually):
   - 3.1. Standard stacks shall (at least 1-3*):
     - 3.1.1 Include images from the surface of the corneal layer to the dermis where resolution is lost.
     - 3.1.2 Captures at the following locations as applicable:
       - Lesion center
       - Where lesion appears different from center (clinically, on dermatoscopy or on RCM)
       - Diagnostic or concerning structures on dermatoscopy or RCM*
       - Include normal background surrounding skin*

Part B. Recommended automated mosaic cube settings for capturing basic image set

1. General cube capture settings:
   - 1.1. Establish z-depth 0 at the surface of the corneal layer
   - 1.2. Select depth of the first mosaic approximately 10 μm below the surface (also see B4, Table I)
   - 1.3. Select number of mosaics: 4-6
   - 1.4. Select spacing of mosaics as per site, age, and lesion specifications noted below (B2-B4, Table I)
2. Body site–specific cube mosaic spacing:
   - 2.1. For body areas (except in elderly, see 3 below), set mosaics 30 μm apart.
   - 2.2. For facial areas (all ages, unless very thinned skin, see 3 & 4, Table I), set mosaics 20 μm apart
3. Age-specific mosaic spacing:
   - 3.1. For all body sites in the elderly, set mosaics 20 μm apart
   - 3.2. For elderly with very thin skin, mosaics may need closer spacing.
4. Lesion and background skin considerations for depth of the first mosaic and mosaic spacing:
   - 4.1. For lesions on very atrophic (thinned) skin,
     - Depth of the first mosaic may need to be decreased
     - Mosaic spacing may need to be decreased
   - 4.2. For lesions where additional mosaics are recommended (see Table II, part B2 and B4; Table III, Part A)
     - Depth of the first mosaic may need to be increased or decreased
     - Mosaic spacing may need to be increased or decreased

RCM, Reflectance confocal microscopy.
*Consensus agreement was not achieved for these recommendations.

could would capture these features intuitively and, therefore, recommendations were not needed. A future study on RCM features warranting stacks (Table III, part B) in the setting of asynchronous/remote interpretation may be valuable. It must be made clear that these recommendations do not require the technicians to know how to diagnose tumors based on RCM. The referring physician must provide the suspected diagnosis to guide additional imaging captures and considerations. The intention of these recommendations is to minimize the responsibility and clinical decision-making process of the technician.

Dermatoscopic images are provided with the RCM images by tissue-coupled devices to help the technician navigate the lesion. Although many agreed that basic dermatoscopy and RCM training requirements for technicians would be ideal, this is not feasible for international recommendations given the variability of experience and available

Table II. Recommendations regarding session maps and procedures to overcome site- and lesion-related imaging challenges and additional captures

Part A. Session maps

1. A session map shall be captured and provided to the reader for localization of mosaics and stacks relative to each other and within the dermatoscopic image for each lesion.
2. A session map should indicate the location of each mosaic and stack within the coregistered dermatoscopic image and include its number in the order of capture. (See Supplementary Fig 3, *A*)
3. If not automatically captured, the device's integrated annotation software can be used to indicate location and assigned number (as per thumbnails) of each mosaic/stack within the dermatoscopic image.
   - 3.1. For lesions requiring >1 basic image set captured within a single RCM placement, the session map clearly defines locations of all mosaics to ease interpretation and mapping (if needed).
4. For lesions requiring >1 RCM window placement (removing and relocating imaging window) due to a lesion size of >8 mm (see Table II Part B and Supplementary Fig 3, *B* and *C*)
   - 4.1. A session map should be created for each RCM placement as per Table II, Part A3 above.
   - 4.2. A supplementary diagram, preferably on a photograph of the lesion, indicating the locations of each RCM placement shall be provided to the reader along with all session maps.

Part B. Recommended procedures to overcome site- and lesion-related challenges

1. For lesions >8 mm in diameter (>1 RCM placement):
   - 1.1. Center the first image set on the portion with the largest diameter, considering a mosaic size of 8 mm^2.
   - 1.2. Capture additional basic image sets (mosaics and stacks, see Table I) to cover majority of the lesion.
   - 1.3. Minimum 10% overlap recommended between each basic image set.
   - 1.4. Adjacent nonlesional skin must be present in at least 1 of the basic image sets.
2. When imaging surface is not flat due to contoured body site or lesion surface elevations/concavities
   - 2.1. Mosaics should begin at the top of the most elevated lesion area.
   - 2.2. Additional mosaics may be required to capture each anatomic level throughout the entire lesion.
   - 2.3. Separate basic image sets, adjacent smaller overlapping or in same location, with z-depth 0 reset at the surface of the corneal layer in the less elevation portion of the lesion between sets, may be most efficient.
   - 2.4. Any mosaics that appear tangential should be discarded, and strategy 2.3 in Table II part B above should be employed.
3. Ulcerated, crusted, or eroded lesions[12-14]
   - 3.1. Shall be imaged with caution as ulcers, crusts, and erosions can cause significant backscatter of light, resulting in dark shadows below, which may preclude evaluation of underlying lesion.
   - 3.2. If the ulcer/crust/erosion covers >20% of the lesion, additional stacks/mosaics captured at the erosion's border and/or strategies used for nonflat lesions (Table II part B2 above) may be required to ensure that the lesion is adequately imaged.[13]
   - 3.3. Ulcers/crusts/erosions covering >50% of the lesion have a high risk of missing diagnostic information using RCM; thus, proceeding directly to biopsy is recommended.
4. For very thickened/raised lesions[12,14]
   - 4.1. Mosaic cube setting may need to be adjusted: increased depth of the first mosaic, increased spacing between mosaics.
   - 4.2. 4 representative mosaics with good resolution at different z-depths shall be captured even if some represent duplicate anatomic levels. (Note that resolution may be lost before all basic set anatomical levels are captured)
   - 4.3. A 5th mosaic with decreased resolution may be included to capture deeper architecture.

RCM, Reflectance confocal microscopy.

training. An understanding of dermatoscopy and basic RCM features has thus been suggested but not recommended.

The group acknowledged that the number, location, and type of images needed for accurate diagnosis depend both on lesion characteristics and the technician's skill and ability to identify key dermatoscopic and RCM features. Therefore, a minimum image set of 4 mosaics (as required by current US CMS reimbursement code) sufficiently capturing the required anatomic levels was deemed acceptable if achievable by the technician. The use of an automated imaging cube should accelerate this process. Additional mosaics or stacks were recommended only in situations where the group determined that they may provide information that could impact management. It must be observed, however, that a recommended

Table III. Recommended additional captures based on suspected diagnosis and reflectance confocal microscopy features

Part A. Additional captures based on suspected diagnosis provided by the referring physician

Although the technician is not expected to perform bedside diagnostics, to supplement the basic image set, the following additional captures may be performed based on the suspected diagnosis of the referring physician.

1. Squamous cell carcinoma in situ/Bowen's disease vs actinic keratosis
   - 1.1. 2 additional epidermal mosaics at different z-depths are recommended: 1 in the corneal layer and 1 in mid epidermis.
   - 1.2. If the lesion is very hyperkeratotic or thickened, see Table II, Part B4.
2. Invasive squamous cell carcinoma/hypertrophic actinic keratosis:
   - 2.1. At least 2 good-quality mosaics of the dermis are recommended if possible, with 1 including the reticular dermis (see Table II, Part B4)
   - 2.2. An additional mosaic is recommended: 1 in the corneal layer
   - 2.3. If the lesion is very hyperkeratotic or thickened, see Table II, Part B4.
   - 2.4. It should be considered, however, that RCM cannot confirm or exclude dermal infiltration with sufficient certainty and confidence, but determining lesion depth may aid in management.
3. Basal cell carcinoma
   - 3.1. More sampling of the epidermis and dermis is recommended to avoid missing the lesion.
   - 3.2. 2 additional mosaics are recommended: 1 in the corneal layer and 1 in the reticular dermis.
   - 3.3. Ensuring good-quality mosaics at the corneal layer, upper mid epidermis, lower epidermis, DEJ, papillary dermis, papillary/reticular dermis is ideal, if possible.
4. Lentigo maligna (<3 cm)
   - 4.1. If the epidermis is atrophic (thin), mosaic cube setting may need adjusting (see Table I, Part B4.2):
     - Decrease depth of the first mosaic to 5 μm
     - Decrease spacing between mosaics to 10 μm
   - 4.2. If DEJ is very flat, additional mosaics may be needed to ensure that the lesion's entire DEJ is captured
   - 4.3. An additional mosaic of the mid epidermis to thoroughly evaluate for pagetoid cells is recommended.
   - 4.4. Additional stacks that follow hair follicles/adnexal structures (especially if they contain bright and/or radiating bright cells) are recommended.[15]
   - 4.5. If differential diagnosis includes any keratosis or carcinoma, add 1 mosaic of the corneal layer.
5. Melanoma
   - 5.1. An additional mosaic of the mid epidermis to thoroughly evaluate for pagetoid cells is recommended.
   - 5.2. If differential diagnosis includes any keratosis or carcinoma, add 1 mosaic of the corneal layer.

Part B. RCM features warranting additional mosaics or stacks if identified (requires more advanced skills)*

If the technician can recognize RCM tumor features, the following structures should be captured if present.

1. Areas with disorder/chaos/abnormality compared to the rest of the lesion.[6,7,16]
2. Medusa sign (bright reflective dendritic or spindled cells radially arranged around a follicle or duct, creating what looks like a Medusa head).[13,15]
3. Numerous large bright atypical cells, large, round, spindled, dendritic or pleomorphic.[6,7,14,16,17]
4. Pagetoid cells.[6,7,14,17]
5. Nests, especially if cerebriform or atypical cells are present.[6,7,14,17]
6. Basaloid tumor island/nest/silhouette.[13,16,17]
7. Areas where the lesion bulges into the dermis
8. Keratin pearls[13]

DEJ, Dermoepidermal junction; *RCM*, reflectance confocal microscopy.
*Consensus agreement was not achieved for this recommendation.

maximum number of images have been included for each scenario to ensure procedure feasibility by optimizing time for the patient, technician, and reader.

It is also important to reiterate that the inherent limitations of RCM may not be overcome by these recommendations. Although RCM diagnostic features have been described to help differentiate between keratinocytic cancer subtypes,[20,21] the limited imaging depth and loss of resolution with depth means that analysis of deeper components of lesion may not be achievable with RCM.[3,9] The recommendations of additional captures are designed as a layer of quality control to aid in differential diagnosis and guide management decisions (Tables I, part A 2.2, and III, part A 2.4 and 3.1). For example, if there are features of actinic keratosis in the epidermis, but tumor is present in the reticular dermal mosaics, then an underlying carcinoma cannot be excluded, and a

punch biopsy should be recommended.[12,13] In this example, the reticular dermal mosaic may decrease the likelihood of a false negative diagnosis and, by indicating the lesion-specific appropriate biopsy type, decrease the risk of a nonrepresentative biopsy specimen transecting the lesion's base. Once again, the recommendations have been developed to encourage the technician and clinician to better discern which lesions may be imaged and how to best image a lesion to ultimately assist in their next steps in management.

CONCLUSIONS AND FUTURE DIRECTIONS

In an effort toward establishing quality standards for image acquisition, these recommendations for in vivo imaging of cutaneous tumors using tissue-coupled RCM have been proposed. The article, Supplementary Figures 1 to 3, and Supplementary Tables I to III (available via Mendeley at https://data.mendeley.com/datasets/kgv9w26ckg/1) are intended to serve as a training tool and reference. Moreover, they may be used as a scaffold for the development of standardized imaging technician certification programs, bridging a needed gap until such programs become available. These recommendations should be considered a living document to be revised according to real-life experience, be it anecdotally or through multicenter trials. Developments such as artificial intelligence–assisted dermoepidermal junction detection and image quality assessment may further facilitate image acquisition in the future.[22,23]

The development of recommendations for image acquisition is one component of the larger picture of quality assurance. Data transfer and sharing recommendations are being developed for RCM according to the Digital Imaging and Communications in Medicines standard. Other components of quality assurance, including health privacy and data/record storage, are part of the broader scope of professional obligation and should be adhered to using established local protocols.

Conflicts of interest

Author Alessi-Fox is a former employee of, currently serves as a consultant to, and holds equity in Caliber Imaging and Diagnostics Inc, the company that manufactures and sells the VivaScope confocal microscope. Dr Gonzalez is a former scientific advisory board member of Caliber ID. Drs Ho, Gill, Grant-Kels, Schwartz, Pellacani, and Guitera have no conflicts of interest to declare.

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