Through the looking glass: Basics and principles of reflectance confocal microscopy

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Reflectance confocal microscopy (RCM) offers high-resolution, noninvasive skin imaging and can help avoid obtaining unnecessary biopsy specimens. It can also increase efficiency in the surgical setting by helping to delineate tumor margins. Diagnostic criteria and several RCM algorithms have been published for the differentiation of benign and malignant neoplasms. We provide an overview of the basic principles of RCM, characteristic RCM features of normal skin and cutaneous neoplasms, and the limitations and future directions of RCM. (J Am Acad Dermatol 2015;73:276-84.)

Key words: basal cell carcinoma; cutaneous oncology; melanoma; noninvasive imaging; reflectance confocal microscopy; squamous cell carcinoma; tumors/neoplasm.

In this rapidly developing medical/technological era, noninvasive imaging devices have emerged as useful tools for diagnosing cutaneous neoplasms. Reflectance confocal microscopy (RCM) provides high-resolution, noninvasive imaging and can be used as an adjunct to the management of skin cancers. Herein, we describe for the general dermatologist the basic principles of RCM, detail its potential applications and current limitations, and discuss RCM features of both normal skin and cutaneous neoplasms.

Abbreviations used:

BCC: basal cell carcinoma
DEJ: dermoepidermal junction
RCM: reflectance confocal microscopy
SCC: squamous cell carcinoma

FUNDAMENTALS OF REFLECTANCE CONFOCAL MICROSCOPY

In vivo RCM is a noninvasive optical imaging technique that provides high-resolution images of skin. RCM relies on a low-power laser that emits near-infrared light (830 nm). To attain a lateral resolution of about 1 μm, RCM allows only light back-reflected from a desired focal point within the skin to pass back through a gating pinhole and enter the detector. The basic RCM optical section image is comparable to high magnification histopathology at about $\times$30; the advantage of RCM compared to histopathology is that optical sections can be obtained in vivo, with no disruption of the skin. Output images are horizontal and parallel to the surface of the skin, visually sectioned in a manner resembling histopathologic specimens in Mohs micrographic surgery.[1] RCM can image the epidermis and papillary dermis but is limited to an imaging depth of about 200 μm.

The wide-probe RCM (VivaScope 1500; CaliberID, Rochester, NY) stitches optical sections into larger mosaic images, allowing a field of view of 8 $\times$ 8 mm. The recent introduction of the handheld RCM (VivaScope 3000; CaliberID) has enabled imaging of lesions on concave surfaces, which are typically difficult to image using the bulky

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Supported by the European Commission Marie Curie FP7 Reintegration Grant to Dr Scope (PIRG07-GA-2010-268359). Dr Rabinovitz has received equipment and funding for a fellowship program from Lucid Inc.

Dr Rabinovitz is an investigator in a study coordinated by Lucid Inc, manufacturer of a commercial confocal microscope. The other authors have no conflicts of interest to declare.

Accepted for publication April 23, 2015.

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Published online June 4, 2015.
0190-9622/$36.00

http://dx.doi.org/10.1016/j.jaad.2015.04.047

wide-probe RCM. Because of its narrow probe, however, the handheld RCM has a field of view limited to 1 × 1 mm.[2] In its current form, the dimensions of the wide-probe RCM imaging station are 30 × 23 × 54 in (76.2 × 58.4 × 137.2 cm); this device can be stowed at the corner of a standard examination room and can be mobilized between examination rooms.

Traditionally, RCM images obtained in the dermatologic setting are black and white, and patterns visualized stem from differences in the reflection of various tissue structures. Highly reflective structures appear bright/white, while nonreflective structures appear dark. Melanin produces the strongest contrast (reflection index = 1.7).[3] The high magnification and resolution of RCM, coupled with the strong reflectivity of melanin, allow for the recognition of individual cells, such as melanocytes and pigmented keratinocytes. Melanin-containing histiocytes (melanophages) are also easily seen on RCM. Keratin is another strong endogenous reflector (reflection index = 1.5), which allows for imaging of keratinocytic neoplasms. In addition, collagen and inflammatory cells strongly reflect RCM light and appear bright in RCM images.[4]

CAPSULE SUMMARY

- Reflectance confocal microscopy provides high-resolution noninvasive skin imaging.
- This review describes the basic principles of reflectance confocal microscopy, its applications in cutaneous oncology, and its limitations.
- As reflectance confocal microscopy continues to evolve, its use in clinical practice can potentially increase a dermatologist's efficiency and diagnostic capability.

POTENTIAL CLINICAL APPLICATIONS OF REFLECTANCE CONFOCAL MICROSCOPY

RCM can be used as an adjunctive diagnostic tool for lesions that display equivocal clinical and dermoscopic features and can increase the physician's and patient's confidence about whether a biopsy specimen is mandated, especially in cosmetically sensitive areas or in anatomic sites from which biopsy specimens are difficult to obtain. RCM has been reported in the bedside evaluation of the oral and genital mucosa[5] and eyelid tumors,[6] with a reported sensitivity of 100% and a specificity of 69.2% for skin cancers on the eyelid as a whole, including basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma.

In the setting of dermatologic surgery, RCM allows for the preoperative diagnosis of skin cancer and can also define surgical margins before excision or between stages of Mohs micrographic surgery.[7-20] According to 1 study, RCM has a sensitivity of 96.6% and a specificity of 89.2%, with a positive predictive value of 93.0% and a negative predictive value of 94.7% for detecting BCCs during Mohs micrographic surgery.[15] Another study cites the sensitivity and specificity of detecting BCCs as 86% and 99%, respectively.[16] There is also reported benefit in using RCM to delineate poorly defined melanocytic lesions, such as lentigo maligna.[21] Of note, RCM is helpful only when neoplastic aggregates are located in the epidermis or papillary dermis; in cases of recurrent BCC or SCC, the neoplastic aggregates are often located in the reticular dermis and may be out of the range of detection of RCM. In such cases, RCM can be used to confirm the presence of a tumor, but a negative RCM examination cannot rule out the possibility of recurrence.

Another indication for RCM is the monitoring of an individual's response to nonsurgical therapies. RCM has been used to monitor the treatment efficacy of imiquimod for actinic keratoses,[22] BCC,[23] and lentigo maligna,[24] and the effect of cryotherapy for superficial BCCs.[25] When BCCs were treated with imiquimod, RCM revealed tumor clearance or a reduction in tumor size, with results corresponding well to histopathologic findings.[23] Again, caution should be taken when interpreting a negative result on RCM, because neoplastic aggregates may be present deeper than the imaging range of RCM. Regular follow-up skin examinations are indicated to assess for recurrent neoplasms.

BASIC TERMINOLOGY AND REFLECTANCE FOCAL MICROSCOPY FINDINGS

Obtaining the correct diagnosis with RCM requires some level of training and experience, and RCM is operator-dependent, as is any morphology-based diagnostic modality. Nevertheless, a few simple algorithms have been designed for the diagnosis of benign and malignant neoplasms, and consensus terminology for RCM has been published.[26]

Normal skin features on RCM are presented below. Please see Table I for a summary.

Suprabasal epidermis

The stratum corneum appears as a highly refractive surface with visible furrows that represent