In Vivo Diagnosis of Skin Cancer through Reflectance Confocal Microscopy

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Introduction

In vivo reflectance confocal microscopy (RCM) is an FDA approved non-invasive optical imaging technique that provides high-resolution cellular images of the skin. This new technology assists the dermatologist in the early diagnosis of skin cancers, thereby reducing the number of unnecessary biopsies performed and improving the malignant to benign biopsy ratio. In 2016, this new technology was awarded a CPT (Current Procedural Terminology) code by the American Medical Association; clinicians can now offer an RCM examination to their patients, while being reimbursed for their effort.

Briefly, RCM is based on a low-power laser, which emits near-infrared light that is projected through a lens system into the skin. The light is backscattered by microscopic tissue components in the epidermis and dermis (e.g., melanin and keratin are strong back-reflectors of RCM light). Only light that returns from a small focal point in the skin will enter the RCM detector through a gating pinhole; all light from outside the desired focal point will be rejected. The RCM instantaneously scans a 500μmX500μm area of tissue in the horizontal plane to produce an en face high-resolution image of tissue (termed an "optical section"). The images appear in black and white – tissue structures that backscatter light will appear bright, while the background skin will appear dark. The resulting optical section is comparable to high magnification (about 30x) histopathology, except that the optical "tissue slides" section the skin parallel to the skin's surface, appear in black and white, and are obtained *in vivo* completely non-invasively. RCM provides cellular detail of the epidermis and papillary dermis down to a depth of about 200μm. The RCM device also incorporates a software that stitches individual optical sections into mosaic images, allowing a field of view of up to 8X8mm (akin to 3-4x low-magnification histopathology). Additionally, RCM has an automated "stacking" function, which allows acquisition of sequential individual optical sections at a particular site in the lesion from the stratum corneum to the papillary dermis.

Previous articles and book chapters have outlined the features seen with RCM imaging of normal skin [1-3]. The stratum corneum appears as a highly refractive surface with visible furrows representing the skin folds. The stratum granulosum and stratum spinosum demonstrate a "honeycombed" pattern, with regular spacing between the keratinocytes and bright cytoplasm and dark oval nuclei. Keratinocytes are noted to diminish in size (from 35 μm to 15 μm) as they approach the basal cell layer. The basal layer appears as aggregates of bright cells corresponding to the horizontal sectioning at suprapapillary plates (described as "cobblestone") or in a circular pattern (termed "edged papillae") around a dark papillary dermis corresponding to horizontal sectioning of dermal papillae. The papillary dermis shows fibrillary collagen bundles and blood vessels, in which blood cells can be seen coursing through the vessels, during real-time imaging.

Images can be read immediately at the bedside and an RCM diagnosis can be rendered to the patient in real-time. Alternatively, RCM images may be transmitted via telemedicine (tele-RCM), using a store-and-forward technique, to an expert who can interpret the images and produce a structured report, similar to a dermatopathology report [4]. The expert reader may receive patient demographic and anamnestic information, a digital dermatoscopic image of the lesion, and a set of RCM images including three to four mosaic maps of the suprabasal epidermis, basal epidermis / dermal-epidermal junction (DEJ), and papillary dermis, as well as image stacks at various foci of the lesion. As in all telemedicine scenarios, a secure server that safeguards information with DICOM (Digital Imaging and Communications in Medicine) and HIPAA (Health Insurance Portability and Accountability Act) compliant security in the United States is required. Utilizing a cloud-based

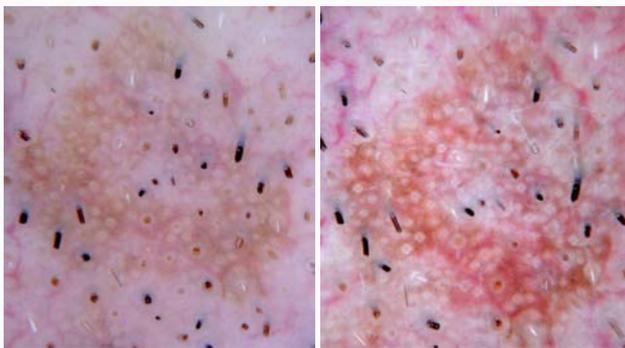**Figure 1:** Tan macule on patient's chin.

Figure 2: Contact non-polarized (left) and contact polarized (right) dermoscopy revealing white circles and keratotic plugs.
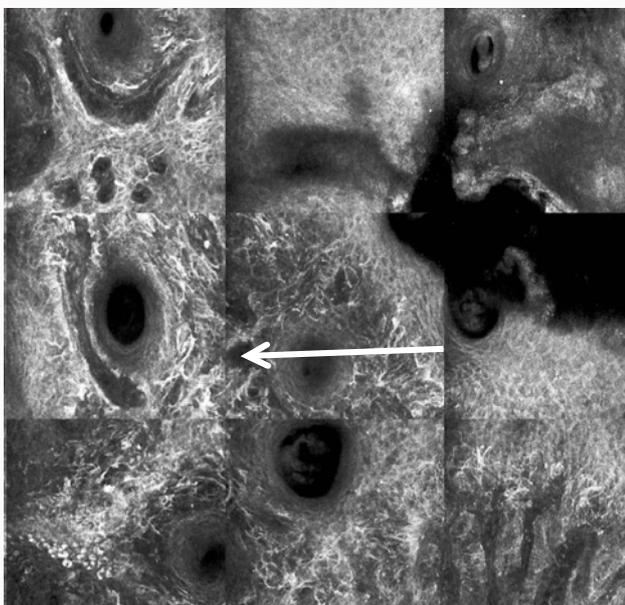

Figure 3: RCM revealing dendritic melanocytes at the epidermis and extending down follicular infundibula.

server and high-speed internet connection (100 mbps), the expert reader can view the images on a high-resolution computer monitor, a tablet, or even mobile phone. Appropriate software affords the reader the ability to navigate through the images, zooming in and out, reminiscent of the functionality of a light microsocpe.

A meta-analysis of the diagnostic accuracy of RCM for melanoma identified a sensitivity of 93% and a specificity 76% [5]. When is it appropriate to apply RCM? RCM is currently best used for the evaluation of pigmented (whereby the source of pigment is melanin, e.g., melanocytic neoplasms) or keratinocytic lesions (e.g. basal cell carcinoma and squamous cell carcinoma). RCM can be used in lieu of an initial diagnostic shave biopsy for lesions that are clinically or dermoscopically equivocal for diagnosis; for example, a patient may be reluctant to have a superfluous biopsy done in cosmetically sensitive areas like the face or in sites that are prone to hypertrophic scars. A clear-cut RCM finding of skin cancer will increase the clinicians' confidence in the required management, and will likely convince the patient to proceed to surgical therapy. On the other hand, an unnecessary biopsy and scarring will be saved if the lesion proves to be benign. At our institutions, we have found RCM to be also useful in patients with many atypical nevi. RCM has been shown to reduce the number of biopsies we need to perform by about 50%, resulting in an improved malignant-to-benign ratio in the biopsies we do perform. Indeed, studies have demonstrated that RCM was able to greatly reduce the number of lesions needed to excise to identify one skin cancer [6,7]. Finally, we have also used RCM to non-invasively monitor the response of skin cancer (e.g., facial lentigo maligna) to imiquimod or other topical chemotherapeutic agents [8].

Clinical Example of Use of RCM in Clinical Practice

Case 1

A 54-year-old man with a past medical history of facial melanoma on sun-damaged skin noticed a new enlarging pigmented lesion on his chin. Examination showed a 6 mm tan macule (Figure 1), and dermoscopy demonstrated white circles and keratotic plugs (Figure 2). The patient was very concerned about scarring at this site. Based upon dermoscopy, the dermatologist considered Bowen's disease as a probable diagnosis and hence contemplated treating the lesion with 5% Fluorouracil (5-FU) topically. To secure the diagnosis prior to topical therapy, RCM was performed. However, RCM revealed many large, bright dendritic cells (compatible with atypical melanocytes) within the suprabasal and basal epidermis and extending down the follicular infundibula (Figure 3); the RCM findings were consistent with the diagnosis of melanoma on sun-damaged skin. An excision demonstrated atypical melanocytes within the epidermis, along the DEJ and extending down follicular infundibula, confirming the diagnosis of melanoma in situ (Figure 4). By using RCM in this case, we were able to confidently proceed directly to a definitive excision without performing an initial shave biopsy, as well as to avoid treating the patient inappropriately with topical 5-FU.

Case 2

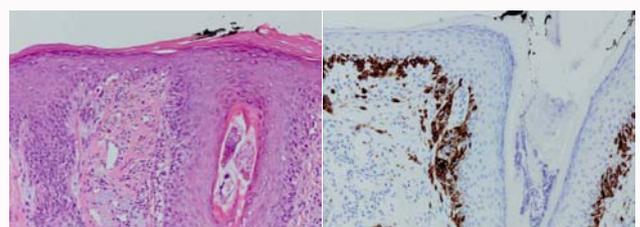A 66-year-old man with a history of melanoma and non-

Figure 4: Histopathology demonstrating a typical melanocytes at the dermal-epidermal junction with hematoxylin and eosin staining (left) and confirmed with HMB-45 immunoperoxidase staining (right) consistent with melanoma in situ on sun-damaged skin.
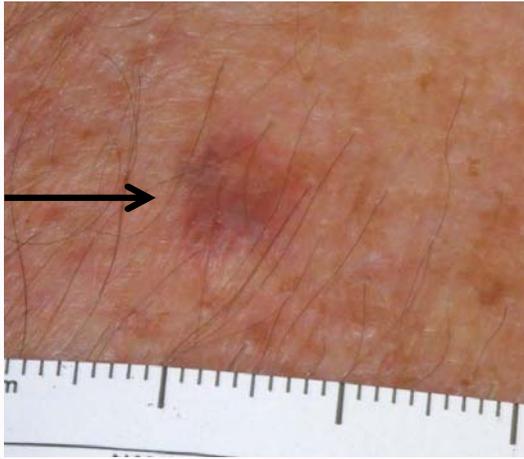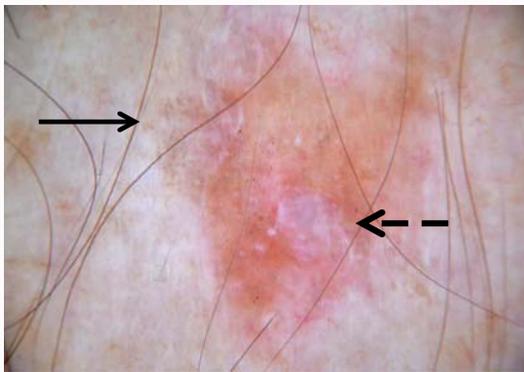
Figure 5: Pink brown papule of the right arm.

Figure 6: Dermoscopy demonstrated focal gray granularity (arrow) and a shiny white area (broken arrow).
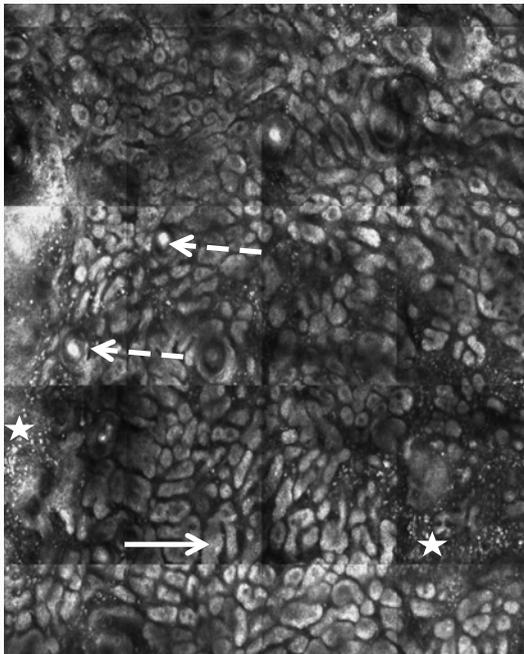

Figure 7: RCM demonstrates elongated cords with bulbous tips (white arrow) and corneal cysts (dashed white arrow) in association with bright plump-bright cells and bright dots (star). These are RCM features of lichen planus-like keratosis.

melanoma skin cancers developed a new pink brown 6 mm papule of the right arm (Figure 5). The clinical differential diagnosis included an atypical melanocytic nevus, melanoma, lichen planus- like keratosis (LPLK), or a pigmented SCC. Dermoscopically (Figure 6), the lesion exhibited focal gray-dot granules (suggestive of melanophages in the papillary dermis) and shiny white structures (also known as the chrysalis sign, suggestive of superficial dermal fibrosis). RCM imaging at the DEJ level demonstrated elongated cords with bulbous tips of the epidermis, as well as cornal cysts (Figure 7). There were some bright plump-bright cells and bright dots (compatible with an inflammatory cell infiltrate) seen within the superficial dermis. These findings confirmed the diagnosis of a lichen planus-like keratosis. The patient was reassured the lesion is benign and that no biopsy is necessary, sparing him discomfort and unnecessary scarring.

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