Should reflectance confocal microscopy be the gold standard for basal cell carcinoma diagnosis?

"*Reflectance confocal microscopy is sufficiently developed to enable real-time diagnosis of basal cell carcinoma with an accuracy and sensitivity almost comparable to histological analysis.*"

KEYWORDS: basal cell carcinoma ▪ confocal reflectance microscopy ▪ noninvasive microscopy ▪ noninvasive monitoring ▪ non-melanoma skin cancer ▪ virtual biopsy

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Reflectance confocal microscopy (RCM) is a novel application of scanning confocal microscopy that generates high spatiotemporal resolution images of the superficial layers of the skin in real time, performed by directly applying a confocal scanning head to the skin. Illumination is provided by infrared laser sources that generate low energy, high penetrance photons. Different structures provide high contrast in the skin, most notably melanin-containing cells and structures, for example, melanocytes and melanosomes, among others [1]. Although penetration should still be improved (current setups enable depths of less than 0.3 mm) RCM is a powerful diagnostic tool owing to its high reproducibility and high resolution, which is comparable to that of conventional histology [2]. Arguably, its most important feature is that it is a bona fide noninvasive technique that permits iterative analytical sampling over time without affecting the area under evaluation. In recent years, RCM has experienced a manifold increase in the number of studies and publications [2,3]. These efforts have yielded a relatively clear and complete picture of the skin under physiological conditions. They have also described the most important RCM features of a number of skin diseases, most notably epidermoid (squamous) carcinoma, basal cell carcinoma (BCC) and melanoma [3]. In this article, I will focus on the most recent findings regarding the imaging and diagnosis of BCC using RCM, provide a brief state-of-the-art regarding BCC microscopic-level diagnosis using RCM and provide insight into current research to improve the use of RCM for diagnosis and evaluation of its therapeutic response.

RCM for the diagnosis of BCC: illuminating skin cancer

BCC is the most frequent skin cancer (it accounts for approximately 80% of skin cancers), especially among individuals of low (pale) skin photo-types. Its etiology is unclear, but includes exposure to UV radiation, genetic modifications and immune suppression [4]. Its clinical appearance and description is circumscribed to four major subtypes of BCC based on the histological growth pattern: superficial, nodular, micronodular and morpheaform [5]. It is a slow growing tumor with a very low metastatic capability. Owing to its frequency (e.g., 1383 cases per 100,000 people in Australia in 2008) and relatively low mortality, BCC remains one of the best characterized types of skin cancer [6]. RCM has been extensively performed in this type of tumor and its major features have been described with detail comparable to that of conventional histology [7]. Hence, RCM reveals the appearance of bright and elongated tumor cells in the basal layer of the epidermis. These cells contain ellipsoidal nuclei, parallel to the overall polarity axis of the cell forming 'palisades' around less polarized tumor cells, which are often referred to as 'tumor islands'. Tumor islands mostly contain basaloid cells and are mostly ellipsoidal in shape. They may also contain very bright, branched cells, which are consistent with melanocytes, and bright granules that correspond to melanosomes [7,8]. 'Clefting' is often observed using conventional histology and corresponds to darker areas filled with mucin. Actinic damage is also associated with this type of tumor. It causes keratinocyte disarray immediately above the basal layer. In addition, inflammatory infiltration and deformation to the underlying blood vessels is apparent. Blood vessels appear dilated (RCM provides enough resolution for width measurements) and leukocyte traffic, visualized as fast motion events inside the capillaries, is heavy in these areas [9]. However, it is important to highlight that none of these features, alone, constitute sufficient evidence to support a diagnosis of BCC.

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Therefore, efforts have been directed to generate a set of diagnostic criteria that provide sufficient sensibility and accuracy to replace conventional histology as the gold standard for BCC diagnosis. Such an initiative yielded five major parameters that comply with reasonable accuracy and sensibility requirements. These pertain to three major morphological categories: tumor cells themselves; vasculature; and overlying epidermis. The specific features originally analyzed were: pertaining to the tumor cell themselves: nuclear polarization and nuclear axial coalignment; regarding the vasculature, observation of leukocyte infiltrates in the lesion and enlarged and/or more numerous blood vessels with horizontal (*en face*) orientation; and corresponding to the epidermis, known as pleomorphism. Separately, these criteria were not particularly reliable; although their individual sensitivity and accuracy were high, they were not exclusive to BCC. However, description of two parameters displayed 100% sensitivity and increased accuracy to 54%; three parameters yielded 94 and 78%, whereas four parameters yielded 83 and 96%, respectively [10]. These and some additional parameters have been recently employed to distinguish between more than 700 equivocal cases with good results [11]. In this case, positive markers of BCC were: polarization and elongation; basaloid nodules; telangiectasia and tortuous blood vessels; and epidermal clefting. Negative markers were diffuse papillae, epidermal disorganization and cerebriform nests (which are typical of melanoma). In this study, RCM imaging of these cases returned values of 100% sensitivity and 88.5% specificity [11].

> "*Reflectance confocal microscopy … generates high spatiotemporal resolution images of the superficial layers of the skin in real time.*"

It is interesting to point out that different clinical studies have revealed minimal differences in terms of RCM diagnosis corresponding to the different subtypes of BCC. One exception is pigmented BCC, which is often mistakenly diagnosed as invasive nodular melanoma. RCM provides a very convenient tool to disambiguate these cases. In the case of pigmented BCC, RCM reveals the presence of the major features described above with minor modifications (e.g., increased numbers of bright, branched melanocytes and bright granular melanosomes interspersed among thick bundles of ruffled collagen and elastin fibers) [12]. Also, other bright structures become visible using RCM. These are

larger than granules but they are not branched, and correspond to infiltrating melanophages [13]. Conversely, nodular melanoma displays cytologic atypia of the dermoepidermal junction. Rete ridges appear disorganized, causing a characteristic deformation in the dermal papillae (nonedged papillae). One of the most striking features of nodular melanoma is the presence of elliptical pagetoid cells and melanocytes inside dermal papillae. In addition, large, reticulated bright shapes called 'cerebriform nests' appear in the dermis [14]. These features are strikingly easy to identify using RCM, thus enabling differential diagnosis with high accuracy.

RCM in BCC therapy follow-up: cleaning the mess

RCM has been used successfully in several therapeutic instances, for example, *a priori* and *in situ* definition of the surgical margins of BCC before Mohs micrographic surgery [15]. In addition to presurgical scanning of the patient, skilled operators can examine the Mohs tissue slices by the surgical table in real time during surgery. This is an ideal readout that informs the surgeon in real time on the cleanliness (i.e., the lack of tumor cells in the sample) of the tumor margin and indicates when and where to finish the surgery. Furthermore, tissue samples examined using RCM can be analyzed *post hoc* using staining-based histology to confirm the results of the RCM survey.

In addition, RCM permits assessing the evolution of the skin in response to nonsurgical treatment in a noninvasive manner. For example, imiquimod is used to promote activation of the immune system, which helps to clear BCC and other skin diseases, for example, epidermoid cancer and warts, among others. Although it is generally used as an adjuvant to surgery, 5% imiquimod yields good results in terms of disease regression and cosmetic appearance. A study used RCM to demonstrate that prior use of imiquimod to treat BCC improved the outcome of Mohs surgery [16]. Other studies have also used RCM to probe the efficacy and long-term effect of other noninvasive therapies, including cryotherapy and photodynamic therapy [17].

A 'deep' look ahead: future perspective

This brief summary clearly illustrates the evolution of the state-of-the art of RCM, which, in the case of BCC diagnosis, has equaled conventional histology. In my opinion, the technique of RCM is sufficiently developed to enable real-time

diagnosis of BCC with an accuracy and sensitivity almost comparable to histological analysis, and without the painful and often disfiguring task of obtaining a tissue sample. Furthermore, the parallel evolution of scanning confocal microscopes, which now enable deeper penetration range, constitute a source of optimism, suggesting that the current technical boundaries of the technique are going to continue to be pushed in upcoming years. Several major challenges remain: the technique is not well known by most practitioners; therefore it is looked upon with a certain degree of skepticism. Therefore, we need to direct efforts and resources to educate technicians and dermatologists in the possibilities and hurdles of the technique, and provide side-by-side training with conventional histology.

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