

ADVANCES IN DERMATOLOGY

Clinical Applications of Reflectance Confocal Microscopy in the Management of Cutaneous Tumors

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Abstract. Reflectance confocal microscopy is a noninvasive tool that allows skin cells and structure to be imaged in real time. The technique has been used to assess benign and malignant lesions and has shown great potential in basic research and clinical dermatology. As might be expected, it also has great potential in longitudinal clinical studies and in the evaluation of dynamic processes such as those that occur after exposure to UV radiation or during the tumor response to noninvasive therapy. This article briefly describes the fundamental aspects and basic principles of reflectance confocal microscopy and discusses its clinical applications essentially in the management of cutaneous tumors. We also consider the limitations of the technique associated with the optical properties of the skin, with instrumentation, and with interpretation of the images.

Key words: reflectance confocal microscopy, cutaneous tumors, virtual biopsy, noninvasive technology.

APLICACIONES CLÍNICAS DE LA MICROSCOPIA CONFOCAL DE REFLECTANCIA EN EL MANEJO DE LOS TUMORES CUTÁNEOS

Resumen. La microscopía confocal de reflectancia es una herramienta de imágenes que permite la visualización de células y estructuras de la piel de forma no invasiva y en tiempo real. Ésta se ha usado para la evaluación de lesiones benignas y malignas, demostrando un gran potencial para aplicaciones en investigación básica y en dermatología clínica. Como cabría pensar, esta tecnología tiene un gran potencial en estudios clínicos longitudinales y en la evaluación de procesos dinámicos como los que acontecen tras la exposición a radiación ultravioleta o durante la respuesta de los tumores a terapias no invasivas. Este artículo discute brevemente los fundamentos o principios básicos de la microscopía confocal de reflectancia, sus aplicaciones clínicas, fundamentalmente en el manejo de los tumores cutáneos, y las limitaciones que la técnica presenta asociadas a las propiedades ópticas de la piel, con la instrumentación y con la interpretación de las imágenes.

Palabras clave: microscopía confocal de reflectancia, tumores cutáneos, biopsia virtual, tecnología no invasiva.

Introduction

For many years, developments in diagnostic imaging techniques have not been applied to studies of the skin, primarily because it is a simple matter to inspect the skin and take samples for histopathology studies. Nonetheless, recent years have witnessed a radical change, given the development of high-resolution noninvasive techniques that aid clinical diagnosis in dermatology. These techniques

include optical coherence tomography, high-frequency ultrasound, and reflectance confocal microscopy. Optical coherence tomography and high-frequency ultrasound are difficult to use in clinical practice, however, because their resolution is limited ($>15 \mu\text{m}$). Furthermore, although these techniques can capture skin sections showing good structural correlation with histology studies, they are incapable of capturing clear images of individual cells.¹⁻⁴ Reflectance confocal microscopy, on the other hand, is a leading-edge technology with sufficient microscopic resolution for diagnostic purposes. This technology, which plays an important role in the development of skin bioengineering, has great potential in dermatology applications, as it enables in vivo and noninvasive histologic diagnoses to be obtained in the presence of the patient. Furthermore, the resolution is very similar to that for

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conventional histology—quasihistologic, in effect. The lateral resolution of less than 1 μm and the vertical resolution of 3 μm to 5 μm are similar to those obtained in routine histology. Although field depth is limited to 250–350 μm , this is sufficient for visualizing the epidermis, the papillary dermis, and the superficial reticular dermis. Image quality is excellent, furthermore. An increasing number of clinical trials have confirmed the correlation between reflectance confocal microscopy and conventional histology findings, pointing to the usefulness of the technique for diagnosing and managing skin cancers.

Basic Concepts and Development

Reflectance confocal microscopy has been undergoing substantial development in the last 15 years, with a key challenge being to develop applications for clinical practice. A number of early studies^{5–7} led to approval of reflectance confocal microscopy by the Food and Drug Administration, the primary regulatory body of the United States of America responsible for health issues. Subsequent development has led to a definition of diagnostic criteria for reflectance confocal microscopy for a number of skin diseases, especially tumors. Particularly useful for training dermatologists in using reflectance confocal microscopy is a recently published atlas developed by our team at the Memorial Sloan-Kettering Cancer Center and containing a description of the confocal characteristics of skin tumors.⁸ Different approaches have clearly demonstrated that the noninvasive diagnosis and treatment of certain kinds of skin tumor is possible.⁹

Reflectance confocal microscopy uses a low-power laser system to illuminate the target lesion in vivo. It functions in a similar way to ultrasonography except that light is used instead of ultrasound. By detecting the light reflected by a microscopic plane less than 5 μm thick, reflectance confocal microscopy can visualize the morphology of skin cells and even their nuclei, thereby allowing differentiation between healthy and atypical cells.⁶ When tissue is observed in real time, dynamic physiological processes can even be observed, including blood flow, leukocyte trafficking in inflammatory processes, and leukocyte migration as a host response to the presence of a tumor.¹⁰ Contrasts in the images reflect the presence of sub-cellular organelles and tissue microstructures. The fact that melanin has a high refractive index makes it a strong endogenous contrast agent and so the technique offers great promise for diagnosing melanocytic lesions such as common nevi, dysplastic nevi, and melanomas.^{5,11}

Clinical Applications

The high resolution of reflectance confocal microscopy in visualizing the skin in vivo means that it has a wide range

of clinical applications, including diagnosis, surgical management of tumors, and evaluation of tumor response to noninvasive treatments. It also has a promising commercial future in cosmetic applications. Below we describe some potential applications.

Biopsy Guidance

Studies conducted by our research team—both at the Harvard Medical School, and, more recently, at the Memorial Sloan-Kettering Cancer Center—have demonstrated that reflectance confocal microscopy is a technique offering great promise in guiding biopsies, particularly in terms of minimizing sampling error, which is an all too frequent problem.^{12–14} A recently published study described the application of reflectance confocal microscopy in the diagnosis of mycosis fungoides,¹⁵ which is challenging because clinical manifestations are highly diverse and pathognomonic findings are not present in the histopathology; consequently, a number of biopsies are generally necessary in order to arrive at a definitive diagnosis. The aforementioned study provided experimental evidence that reflectance confocal microscopy can locate the most suitable biopsy site within a lesion, given its capacity for detecting significant histologic changes in the epidermis and superficial dermis. Other lesions for which reflectance confocal microscopy may help diagnosis are those located in cosmetically sensitive areas such as the face, particularly when the differential diagnosis includes lentigo maligna and lentigo maligna melanoma. Since lentigo maligna melanoma is extremely clinically heterogeneous, with areas that histologically may appear to be solar lentiginosities, taking a sample from the right place is very important,^{16,17} especially as it also avoids conducting biopsies for solar lentiginosities. Another disease for which reflectance confocal microscopy may be useful is atypical nevus syndrome, given that patients with this syndrome typically undergo unnecessary biopsies (some of the nevi turn out to be benign).¹⁸

Demarcation of Poorly Defined Tumor Margins

The ideal treatment for tumors located in the face or scalp is full excision of the tumor along with histologically verified tumor-free margins. Tumors—for example, melanomas such as lentigo maligna, lentigo maligna melanoma, and amelanotic melanoma—often present with poorly defined borders. The complexity of lentigo maligna and lentigo maligna melanoma is clinically challenging; furthermore, lesions are difficult to treat as they typically feature chronic actinic skin damage, making histologic demarcation difficult. When located on the face, such lesions are particularly

problematic, since ensuring adequate margins will clearly have important cosmetic or functional repercussions. A number of preliminary studies have recently been published that demonstrate the potential of reflectance confocal microscopy for presurgical mapping of lentigo maligna melanomas and amelanotic malignant melanomas with poorly defined borders.^{16,17,19} Our Sloan-Kettering research team is currently implementing a large-scale clinical study aimed at confirming this particular application. The procedure consists of conducting a conventional clinical examination of the lesion; margins are then delineated using Wood light and dermatoscopy (which, in some cases, helps detect subclinical manifestations); and, finally, reflectance confocal microscopy is applied to the areas both inside and outside the delineated margins so as to map the lesion borders.

Monitoring of Tumor Response to Noninvasive Treatment

The substantial clinical interest in noninvasive treatments for eliminating cancers has created a demand for technologies that can confirm, with clinical certainty, that the cancer has been cured without the need for biopsy. Noninvasive treatments which have recently received regulatory approval include photodynamic therapy and topical treatment with imiquimod (an immune response modifier), both of which are indicated for actinic keratoses, Bowen disease, and certain kinds of basal cell epithelioma. It is very important that reflectance confocal microscopy can both characterize the initial disease and confirm treatment success. A number of studies undertaken by our Harvard Medical School research team have demonstrated the value of reflectance confocal microscopy in monitoring tumor response to photodynamic therapy and imiquimod treatment,²⁰⁻²² and also the potential offered by reflectance confocal microscopy for detecting residual carcinoma following tumor treatment or biopsies of tumors with involved margins.²³

Limitations

Reflectance confocal microscopy has certain limitations associated with the optical properties of the skin, the imaging device, and the interpretation of images. An important limitation in terms of analyzing certain diseases such as melanoma is that the optical properties of the skin are such that the depth of the reflectance confocal microscopy analysis is limited to the superficial reticular dermis. Most reflectance confocal microscopy devices that are available commercially can easily be applied to nearly all areas of the body and there is even a hand-held device, little bigger than an otoscope, which makes access to specific areas of the body

even easier. Image interpretation, however, is probably the greatest obstacle to more widespread use of reflectance confocal microscopy in clinical practice. To overcome this limitation, a teledermatology server for digital image consultation with experts is being developed under the auspices of Digital Imaging and Communications in Medicine (DICOM), a standard used for the storage, transfer, and processing of medical images. This DICOM application will enable digital images to be sent to an expert (in a way similar to the transmission of biopsies to dermatopathologists), who will return a report to the dermatologist, possibly even before the patient leaves the clinic.

Conflicts of Interest

The author declares no conflicts of interest.

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