New Directions in Dermatopathology

In Vivo Confocal Microscopy in Clinical Practice

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KEYWORDS

• In vivo confocal microscopy • Skin tumors • Inflammatory diseases • Infectious diseases

KEY POINTS

- Reflectance confocal microscopy (RCM) is a novel noninvasive imaging technique that enables the identification of cells and tissues with nearly histologic resolution.
- In the epidermis, keratinocytes are clearly visualized as small polygonal nucleated structures, and epidermal alterations, such as acanthosis, hyperkeratosis, exocytosis, and spongiosis, and others can be easily identified.
- RCM also enables the visualization of dermal structures up to the papillary dermis.
- In melanocytic and epithelial skin tumors, cytologic atypia as well as architectural disarrangement can be visualized, helping in the achievement of a more accurate diagnosis.
- The application in inflammatory and infectious skin diseases showed good correlation with microscopic findings, although inflammatory cell subpopulations cannot be distinguished.

INTRODUCTION

Reflectance confocal microscopy (RCM) represents a new imaging tool that enables the identification of cells and tissues with nearly histologic resolution.[1–3] Although several noninvasive tools have been explored to test their potential application in the clinical field, RCM has emerged as a unique instrument because it can visualize the skin tissue with a resolution that is comparable with conventional histopathology. It allows a horizontal scanning of the imaged tissue, with the advantage of exploring a larger field of view compared with vertical sectioning. Moreover, the horizontal plane offers a perfect correlation with clinical and dermoscopic aspects, which is crucial when dealing with skin tumor diagnosis. In this article, we present the main confocal findings and their correlations with histopathology along with a brief description of confocal applications in the clinic arena.

INSTRUMENTS

The commercially available confocal microscope (VivaScope 1500, Lucid, Rochester, NY) contains a probe (the head of the microscope), which is attached to the skin by using a disposable plastic window, which is in turn taped to a metal ring. A confocal microscope consists of a point source of light, condenser, objective lenses, and a point detector.[1,2] The pinhole collects light emanating only from the in focus plane. The mechanism of bright contrast in RCM is backscattering. In gray-scale confocal images, structures that appear bright (white) have components with high refractive

No conflict of interest to disclose.

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Dermatol Clin 30 (2012) 799–814
http://dx.doi.org/10.1016/j.det.2012.06.012
0733-8635/12/$ – see front matter

index compared with their surroundings and are similar in size to the wavelength of light. Backscattering is primarily governed by the refractive index of the structure compared with surrounding medium. Highly reflective skin components include melanin, collagen, and keratin. The confocal scanning produces high-resolution black-and-white horizontal images (0.5 × 0.5 mm) with a lateral resolution of 1.0 μm and axial resolution of 3 to 5 μm. A sequence of full-resolution individual images at a given depth is acquired and stitched together to create a mosaic ranging in size from 2 × 2 mm to 8 × 8 mm. For inflammatory or physiologic skin conditions, a 3 × 3 mm VivaCube (Lucid, Rochester, NY) composed of 4 mosaics with a 25-μm step is usually acquired, whereas for skin tumor examination, the imaging should include the entire lesion. Besides the horizontal mosaic, a vertical VivaStack can be imaged. It consists of single high-resolution images acquired from the top skin surface up to 200 μm, corresponding to the papillary dermis, to obtain a sort of optic biopsy. The VivaStack (Lucid, Rochester, NY) modality is useful for the assessment of the epidermal thickness either in physiologic condition or in the presence of dysfunctional epidermis.

Recently, a handheld RCM has been introduced on the market (VivaScope 3000). This version is a smaller, flexible device, which is useful in areas that are difficult to access (eg, skin folds, ears). Unlike the 1500 version, it has an on-instrument control for laser power, imaging depth, and capture but it does not allow scanning of a large field of view, which is needed, for example, in some tumors to obtain an overview of the architecture. However, it is a promising tool, which can be used for surgical premapping or when multiple site imaging is requested.

MAIN HISTOPATHOLOGIC CORRELATES OF CONFOCAL CRITERIA

Epidermis

The epidermis can be affected by several injuries that lead to different morphologic changes involving the keratinocytes (KCs) or other cells of the epidermis, such as melanocytes.

The epidermal changes are described as phenomenon per se regardless of their relationship with either inflammatory or skin tumors.

In healthy young skin, the epidermis appears as a multilayer tissue with paradigmatic confocal aspects depending on the skin level.[4,5] The stratum corneum appears as a highly refractive surface surrounded by visible skin furrows. At this level, the corneocytes are large, ranging from 10 to 30 μm, polygonal, and without a visible nucleus. Skin furrows appear as dark folds between islands of KCs. In young people, the skin furrows are arranged in a rhomboidal pattern formed by intersecting skin furrows. However, the shape and arrangement of the skin folds strongly depends on the body site (being almost absent on the forehead and well represented on the abdomen) and the individual's age. The stratum granulosum is composed of polygonal KCs presenting a bright and grainy cytoplasm because of the presence of organelles. The KCs cohesively assemble, forming a structure that gives rise to a honeycombed pattern.[5] The contour of the cell is usually brighter than the cytoplasm and perfectly outlined. Pigmented KCs are usually bright cells, small in size and always polygonal, separated by a darker contour (cobblestone pattern), resembling the negative of the honeycombed pattern. In the honeycombed pattern, the KCs appear black with bright contour, whereas in the cobblestone pattern, these cells show a bright cytoplasm because of the high melanin content (ie, brightness) (**Fig. 1**). On the face, a peculiar pattern is caused by the presence of numerous hair follicles that appear as dark round areas (donutlike appearance, H Rabinovitz, personal observation). At the stratum spinosum level, the size of KCs tends to decrease but the cells still have a polygonal shape. The honeycombed pattern is easily observable.

Acanthosis is one of the most frequent findings and it can be observed in several conditions. Histopathologically, acanthosis is defined as diffuse epidermal hyperplasia caused by the increased thickness of the stratum spinosum constituted by the prickle cells.

Because of the horizontal sectioning, only an indirect correlate with acanthosis is feasible on RCM. The granulosum and spinosum layers of an acanthotic epidermis consist of islands of KCs with broadened greyish outlines (**Fig. 2**). Moreover, when using the VivaStack modality, an increased thickness of the stratum granulosum/spinosum can be detected and measured by counting the layers.

Dermoepidermal Junction

Below the spinous layer, there is a single layer of basal cells at the dermoepidermal junction (DEJ).[4] Basal cells are uniform in size and shape but are smaller and more refractive than spinous KCs because of the melanin caps forming bright disks on top of the nuclei. The brightness of KCs is strongly dependent on the skin phototype. Dark skin phototypes show basal KCs that are bright round cells with highly refractive cytoplasm forming a cobblestone pattern at the epidermal level and