Review paper

The use of reflectance confocal microscopy in selected inflammatory skin diseases

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Reflectance confocal microscopy is a modern, non-invasive diagnostic method that enables real-time imaging of the epidermis and upper layers of the dermis with nearly histological precision and high contrast. The application of this technology to skin imaging during the last years has resulted in progress of dermatological diagnosis, providing virtual access to living skin, without the need for conventional histopathology. The presented method potentially has broad application in the diagnosis of skin diseases. This article provides a summary of the latest reports and previous achievements in the field of reflectance confocal microscopy. General characteristics of confocal images in selected inflammatory skin diseases are presented.

Key words: reflectance confocal microscopy, *in vivo* biopsy, non-invasive imaging technique.

Introduction

Reflectance confocal microscopy (RCM) is a modern, non-invasive diagnostic method that enables real-time imaging of the epidermis and upper layers of the dermis with a nearly histological precision and high contrast [1]. It has a wide range of applications, particularly in the diagnosis of neoplastic skin lesions, enabling non-invasive monitoring of treatment [2-6], in examining skin reactions to external factors (e.g. ultraviolet radiation) [7], as well as planning surgical margins in the course of pre- and intraoperative evaluation [8-10]. Reflectance confocal microscopy has also been applied in the study of a number of non-neoplastic skin diseases, especially inflammatory dermatoses. Currently, the histopathological examination is the primary diagnostic tool in dermatology, yet a skin biopsy is painful, leaves a scar and the final diagnosis may require multiple interventions. The use of RCM eliminates these drawbacks by enabling quick and painless skin visualization *in vivo*. The skin is not altered by the procedure, which eliminates the risk of artifacts. All images can be stored electronically; therefore they can be reproduced and compared with successive results, which allows for evaluation of dynamic changes in the skin such as the response to external stimuli or response to treatment [1].

Reflectance confocal microscopy efficacy in the inflammatory dermatoses

Psoriasis

Histopathological features of *psoriasis vulgaris* can be easily visualized by means of RCM. Optical sections of psoriatic plaques show an increase in the diameter of the dermal papilla (> 100 μm) together with an increase in the diameter of papillary blood vessels (Fig. 1) [11]. Parakeratosis is another characteristic feature of the image; it appears as dark cell nuclei within bright cells of the stratum corneum. So-

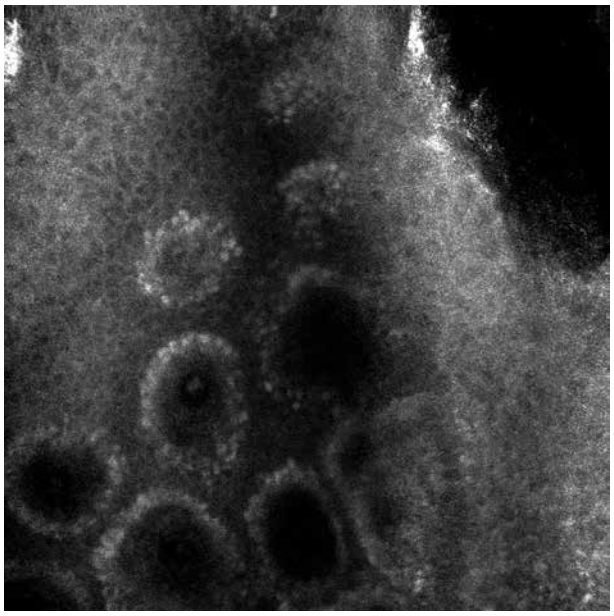

Fig. 1. *Psoriasis vulgaris*. Increased diameter of the dermal papilla and increased diameter of blood vessels in the papillary dermis are visible

called Munro's microabscess can be found in the form of clusters of bright cells, 6 to 30 μm in diameter, located between the corneocytes of the stratum corneum. Going deeper into the epidermis, we observe a significant reduction in thickness or even the lack of a stratum granulosum with simultaneous acanthosis of the stratum spinosum [12, 13]. The thickness of the epidermis in the psoriatic plaques is significantly increased compared to the healthy skin and can be up to 300 μm.

Darier's disease

Darier-White disease is a rare, inherited autosomal dominant skin condition characterized by a disorder of keratinization. González *et al*. [14] presented confocal images of dyskeratosis in the form of 7-10 μm cells in the stratum corneum, some of them containing a pyknotic nucleus, called Darier's grains. Below the stratum corneum the so-called 'corps ronds' 20-25 μm in diameter were found. Additionally, atrophy of the stratum granulosum was observed in the acantholytic area. In the stratum papillare of the dermis, thickening of collagen fibers was visualized.

Lichen planus

In 2012 Moscarella *et al*. [15] published the results of a pilot study of the RCM efficacy in the diagnosis of lichen planus (LP). Large polygonal cells containing grainy cytoplasm, a feature corresponding to hypergranulosis in histology, were found within the

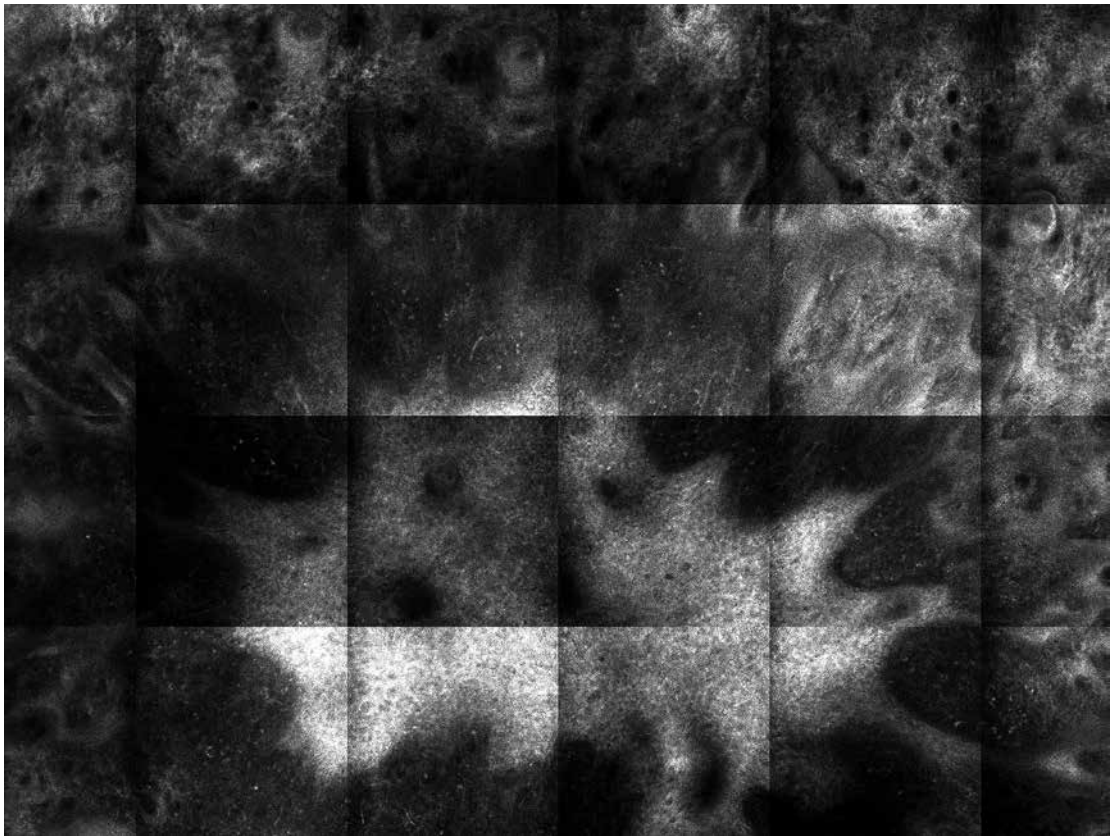

Fig. 2. *Lichen planus*. Disseminated inflammatory cells and necrotic keratinocytes in the surroundings of the lesion

stratum granulosum. The stratum spinosum showed characteristics of moderate spongiosis. Throughout the epidermis numerous inflammatory infiltrates in the form of circular and polygonal light cells were observed. Due to extensive infiltration of inflammatory cells within the dermoepidermal junction, papillary rims were obscured. Dilated blood vessels could be found in the superficial dermis. Although reflectance confocal microscopy may prove to be an important aid to diagnosis of LP, it has not been used in the differential diagnosis of its subtypes (Fig. 2).

Contact dermatitis

Differential diagnosis between allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) is a true challenge due to a high similarity in clinical appearance, histology, and immunohistology [16-18]. Reflectance confocal microscopy has been applied to examine both conditions. Spongiosis is their common feature and in cross-section is visible as increased brightness within the intercellular spaces. Inflammatory infiltrates in the form of bright, round or oval cells with 9-12 μm diameter are observed between the cells of the epidermis. Other common features include epidermal necrosis, perivascular inflammatory infiltrates and an increase in the size and brightness of the basal keratinocytes. Allergic contact dermatitis's main histopathological features, observed also by means of RCM, are the presence of intra-epidermal microbubbles, inflammatory infiltrates and spongiosis. In turn, ICD manifests itself through superficial changes, mainly involving the stratum corneum with a distinct borderline between the healthy and affected skin. Parakeratosis (dark nuclei within bright cells of the stratum corneum) is another characteristic feature of ICD [19, 20].

In 2005 Astner *et al.* [20] conducted a pilot study to determine the sensitivity and specificity of RCM in diagnosing ACD by means of patch testing. Optical sections revealed necrosis of the epidermis, parakeratosis, spongiosis and exocytosis within the stratum spinosum. Preliminary data showed high sensitivity and specificity for the detection of spongiosis and exocytosis in the diagnosis of ACD. The results suggest that RCM can provide valuable data for interpreting the results of patch tests.

Reflectance confocal microscopy was also used to assess the kinetics of CD. It was found that the appearance of lesions was much faster in ICD than in ACD. The appearance of superficial changes in the stratum corneum was apparent a few hours after the application of the irritant, whereas after the application of the contact allergen much less acute changes appeared in the stratum corneum after a considerably longer time [19, 20].

It was also found that hyperkeratosis in ICD is a sensitive parameter of the reaction. There have been several studies on the assessment of ethnic variation in response to the application of irritants [21, 22]. Preliminary studies in populations of Caucasian and African volunteers have shown varying degrees of reactivity within the stratum corneum to cutaneous irritants (black skin appeared to be more resistant to the development of ICD symptoms than white skin). Taking this into consideration, RCM may prove to be an effective tool in CD research, providing a wide range of possibilities, including in the examination of allergens and their concentrations.

Pemphigus vulgaris and pemphigus foliaceus

In the case of patients with bullous eruptions the initial diagnostic test by means of RCM, the opportunity to assess the epidermal detachment threshold and the possibility of performing non-invasive visualization of acantholytic cells are crucial [23]. In 2011, Kurzeja *et al.* [24] published the results of an efficacy study of RCM for the diagnosis of pemphigus vulgaris (PV) and pemphigus foliaceus (PF). Thirty patients (18 with PV and 12 with PF) were included in the research. Intra-epidermal bullae with acantholytic cells were revealed in 47% of PV patients and 59% of PF patients. In most cases, these symptoms were accompanied by inflammatory infiltrates, dilated blood vessels in the papillary layer of the dermis, the loss of the epidermal honeycomb structure as well as detachment of the outer root sheath in the hair follicle. In a significant number of cases, these features could also be observed in the healthy skin adjacent to bullae.

Kurzeja *et al.* have developed criteria for the RCM diagnosis of pemphigus. They are as follows: 1) acantholytic clefts within a lesion, 2) acantholytic clefts in the healthy skin adjacent to a lesion, 3) dilated blood vessels within the papillary dermis of a lesion. Fulfilling two of these three criteria allows the diagnosis of pemphigus to be established. However, this method does not allow one to differentiate between PV and PF.

All in all, RCM seems to be a useful method for the rapid, non-invasive diagnosis of pemphigus. Nevertheless, histopathological and immunological tests remain the gold standard for diagnosis.

Discoid lupus erythematosus

Discoid lupus erythematosus (DLE) lesions in optical sections showed a correlation with the histopathological image [25, 26]. Total architectural disarray of the stratum spinosum was observed with clear spongiosis as well as perivascular and perifollicular inflammatory infiltrates. There was also blurring of the normal

structure of the dermoepidermal junction with numerous inflammatory cells within the dermal papilla. Dilated blood vessels and sclerotic collagen fibers were observed in the dermis (Fig. 3).

Rosacea

Rosacea is a benign inflammatory disease of unknown etiology that occurs more often in women. Its dermatological symptoms are typically localized on the face, with characteristic papules and pustules, as well as dilation of blood vessels. Reflectance confocal microscopy images present increased diameter of pilosebaceous units, numerous enlarged and twisted blood vessels and characteristic perivascular and perifollicular inflammatory infiltrates [27].

Folliculitis

Reflectance confocal microscopy imaging of inflammatory folliculitis presents perifollicular inflammation in the form of numerous bright clusters of granule cells corresponding to a neutrophil. These cells can also be visualized within pustular eruptions. The characteristic elements of the RCM image also include severe spongiosis and dilation of widening blood vessels in the dermal papilla [27].

Viral infections

Reflectance confocal microscopy optical sections of herpes simplex skin infections present large, round, pleomorphic cells with dark cytoplasm, corresponding to keratinocytes with ballooning degeneration and bright circular structures corresponding to multinuclear giant cells. Both cell types tend to form loose aggregates, interlaced with round, bright inflammatory cells [28].

Reflectance confocal microscopy is also used in the evaluation of common warts. The optical sections demonstrate hyperkeratosis and the presence of many round, strongly refractile structures 20-40 μm in diameter with a probable correlation to keratohyalin granule or fragments of the virus in infected keratinocytes [29].

Fungal infections

Dermatophyte infections, despite high prevalence, often pose diagnostic difficulties. The clinical image of a fungal infection is not always clear, and standard mycological examination is time consuming. Reflectance confocal microscopy allows for a rapid *in vivo* diagnosis based on the visualization of hyphae in the form of a network of long, dark, occasionally branched, linear structures [30]. Hyphae could also be demonstrated as highly refracting, bright, linear structures in an *in vitro* examination of a nail sample with a 10% solution of potassium hydroxide [31].

In 2013, Rothmund *et al.* [32] conducted a study to compare the sensitivity and specificity of traditional and new diagnostic tools (RCM, OCT) for testing fungal nail infections. Upon testing a group of 60 patients with nail lesions (50 patients with onychomycosis and 10 controls with other changes of the nail) the sensitivity of RCM was estimated at the level of 79.5% (higher sensitivity was achieved only with PCR and OCT), whereas the specificity was 81% (higher specificity was obtained with PCR, culture and histo-

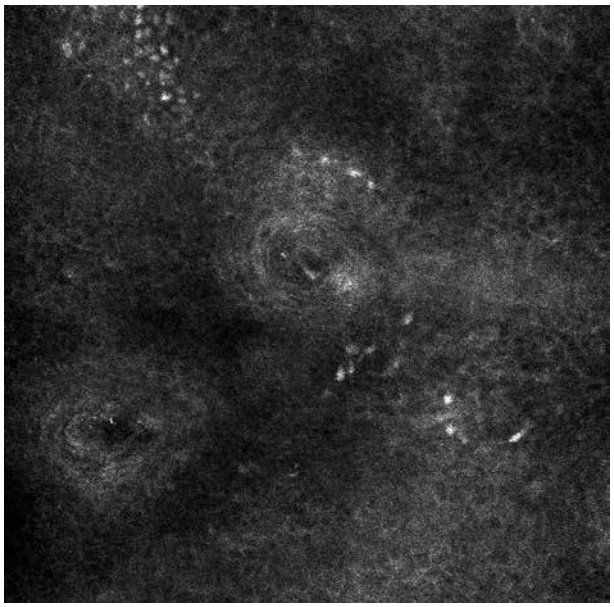

Fig. 3. *Discoid lupus erythematosus*. Perifollicular inflammatory infiltrations

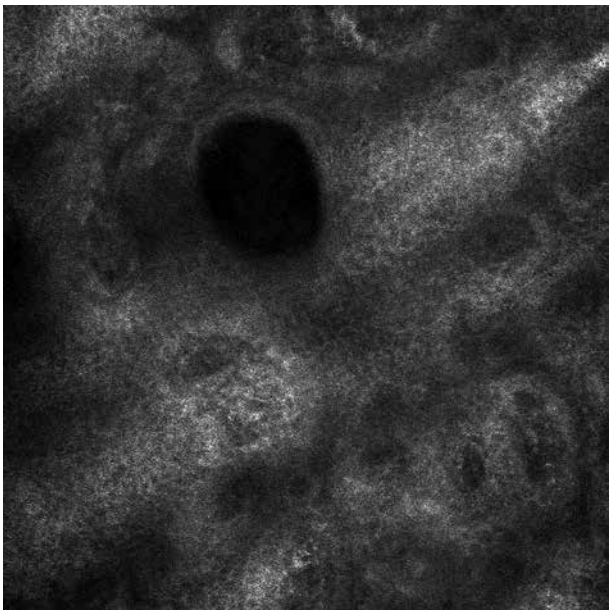

Fig. 4. *Alopecia areata*. Empty lumina with highly refractile material are visible

pathological examination). This suggests that RCM may be useful in the diagnosis of onychomycosis.

Parasitic infections

Longo *et al.* [33] detected by means of RCM a *Sarcoptes scabiei* of an oval structure with two pairs of denticles. This method also permits visualization of tunnels drilled by the female mite within the stratum corneum.

Hair conditions

On an RCM image a hair appears as a long, bright, evenly luminous cylindrical structure. Recent studies have shown that RCM may be a useful auxiliary tool in the diagnosis of hair diseases such as alopecia areata, androgenetic alopecia, or hereditary hair dystrophy, although its superiority over trichoscopy has not yet been demonstrated [34]. Ardigo *et al.* [35] performed an RCM study of stratum spinosum of the epidermis in patients with alopecia areata. The study showed a remarkable decrease of follicular adnexal structures and empty lumina with highly refractile material (Fig. 4) corresponding to the yellow dots seen on trichoscopy.

The advantage of RCM is the possibility to obtain the highest magnification available in non-invasive skin imaging techniques and thus the ability to visualize the hair structure with more detail than by means of trichoscopy. It proves useful in the imaging of hereditary hair conditions, but is limited by the need to attach an adhesive metal ring to the scalp.

Reflectance confocal microscopy limitations and potential solutions

Due to the limited possibility of penetration into the skin (approx. 200-300 μm) reflectance confocal microscopy allows only for the observation of the epidermis, the papillary layer and the upper part of the reticular layer of the dermis. In the case of keratosis, the imaging depth may be even lower due to the increased thickness of the stratum corneum. The application of a different light source and immersion medium could potentially improve the depth of penetration. In addition, grayscale imaging strikingly impedes the recognition of individual organelles and tissue structures. The use of exogenous contrast agents could become a solution.

The high cost compared to the price of conventional microscopes is another challenge limiting the availability of the method at present. The large size, difficult application to uneven surfaces and bulkiness of the device tend to be extremely onerous. Certainly, advances in the technology will reduce the size of the confocal microscope, creating a cheaper, more convenient, widely available, and perhaps even a handheld model.

Limited user-friendliness of the appliance and complicated result interpretation, which in turn requires constant self-education, pose yet another drawback of RCM. At present, there is also the lack of clearly defined diagnostic algorithms for most conditions. The growing reputation of the technology among dermatologists and pathologists will in time lead to the creation of sensitive and specific diagnostic algorithms. Despite the discussed limitations, RCM remains an excellent support for a rapid diagnosis of atypical clinical images, eliminating at the same time the need for time-consuming and expensive biopsies.

Summary and conclusions

Histopathological examination is the gold standard of the diagnosis of skin diseases, yet its invasive nature creates many limitations. Reflectance confocal microscopy is an alternative allowing for non-invasive visualization of the tissue while maintaining high resolution and good contrast. In most cases, benign inflammatory skin diseases are confined to the epidermis and upper layers of the dermis where the accuracy of confocal microscopy is comparable to conventional histopathology. The method is used in both *in vivo* and *ex vivo* scientific research and in clinical practice. Its introduction into everyday dermatological practice can reduce the number of invasive diagnostic biopsies. Presently, RCM remains in the early stages of development, so we are forced to struggle with its many limitations. Further research and increasing popularity of RCM may soon lead to the improvement of the technology and the creation of clearly defined diagnostic criteria.

The authors declare no conflict of interest.

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