Reflectance confocal microscopy for the diagnosis of skin infections and infestations

Rita Pimenta[1] MD, Luís Soares-de-Almeida[1,2] MD PhD, Edith Arzberger[3] MD, João Ferreira[1,2] MD PhD, Paulo Leal-Filipe[1,2] MD PhD, Pedro Mendes Bastos[4] MD, André Laureano Oliveira[1,2] MD

Affiliations: [1]Department of Dermatology, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal, [2]Centro Académico de Medicina, University of Lisbon, Lisbon, Portugal, [3]Department of Dermatology, Medical University of Graz, Graz, Austria, [4]Dermatology Center, Hospital CUF Descobertas, Lisboa, Portugal

Corresponding Author: Rita Pimenta MD, Clínica Universitária de Dermatologia de Lisboa, Hospital de Santa Maria, Avenida Professor Egas Moniz, 1649-028 Lisboa, Portugal, Tel: 351-912199704, Fax: 351-217954447, Email: ritapimenta@campus.ul.pt

Abstract

Reflectance confocal microscopy (RCM) is a noninvasive real-time imaging technique that has been widely used for the diagnosis of skin cancer. More recently, it has been reported as a useful tool for the diagnosis and management of several inflammatory and infectious skin disorders. This article provides an overview of the current available applications of RCM use in cutaneous infections and infestations. PubMed was used to search the following terms in various combinations: reflectance confocal microscopy, skin, hair, nail, infection, parasitosis, mycosis, virus, bacteria. All papers were accordingly reviewed. In most cutaneous infections or infestations, the main alterations are found in the epidermis and upper dermis, where the accuracy of confocal microscopy is nearly similar to that of histopathology. The high resolution of this technique allows the visualization of most skin parasites, fungi, and a few bacteria. Although viruses cannot be identified because of their small size, viral cytopathic effects can be observed on keratinocytes. In addition, RCM can be used to monitor the response to treatment, thereby reducing unnecessary treatments.

Keywords: confocal microscopy, parasitosis, mycosis, bacteria, virus

Introduction

Reflectance confocal microscopy (RCM) is a noninvasive imaging technique that allows real-time, en face visualization of the epidermis and upper dermis, with a near cellular resolution. It has been widely used for the diagnosis of melanoma and non-melanoma skin cancer [1]. More recently, RCM has been reported as a useful tool also for the diagnosis and management of inflammatory and infectious skin disorders.

Confocal microscopy may be particularly suited for the diagnosis of cutaneous infestations or infections as most of the pathologic clues are confined to the epidermis. The high resolution of RCM (1.00µm laterally and 5µm vertically) allows the visualization of most skin parasites and dermatophyte hyphae and conidia [2]. Although viruses cannot be identified because they are too small, diagnoses can be achieved indirectly by visualization of viral cytopathic effects on keratinocytes [3]. Theoretically, some bacteria can also be visualized but so far there are only a few reported cases of the identification of syphilis and secondary bacterial infections [4-6].

For in vivo use, there are two commercially available microscopes: VivaScope 1500® and 3000® (Caliber: imaging and diagnostics, Rochester, NY, USA). The standard device, VivaScope 1500®, allows the scanning of a large area (up to 8×8mm), owing to both the mosaic and stack imaging modalities. However, it generally requires an acquisition time of about 10 minutes per lesion and it cannot be used on small or curved surfaces. The handheld compact camera, VivaScope® 3000, is easier to manipulate, faster to use (1-2 minutes per lesion), and has a smaller tip enabling access to hard-to-reach body areas [7]. Nonetheless, it is limited by a smaller field of view.

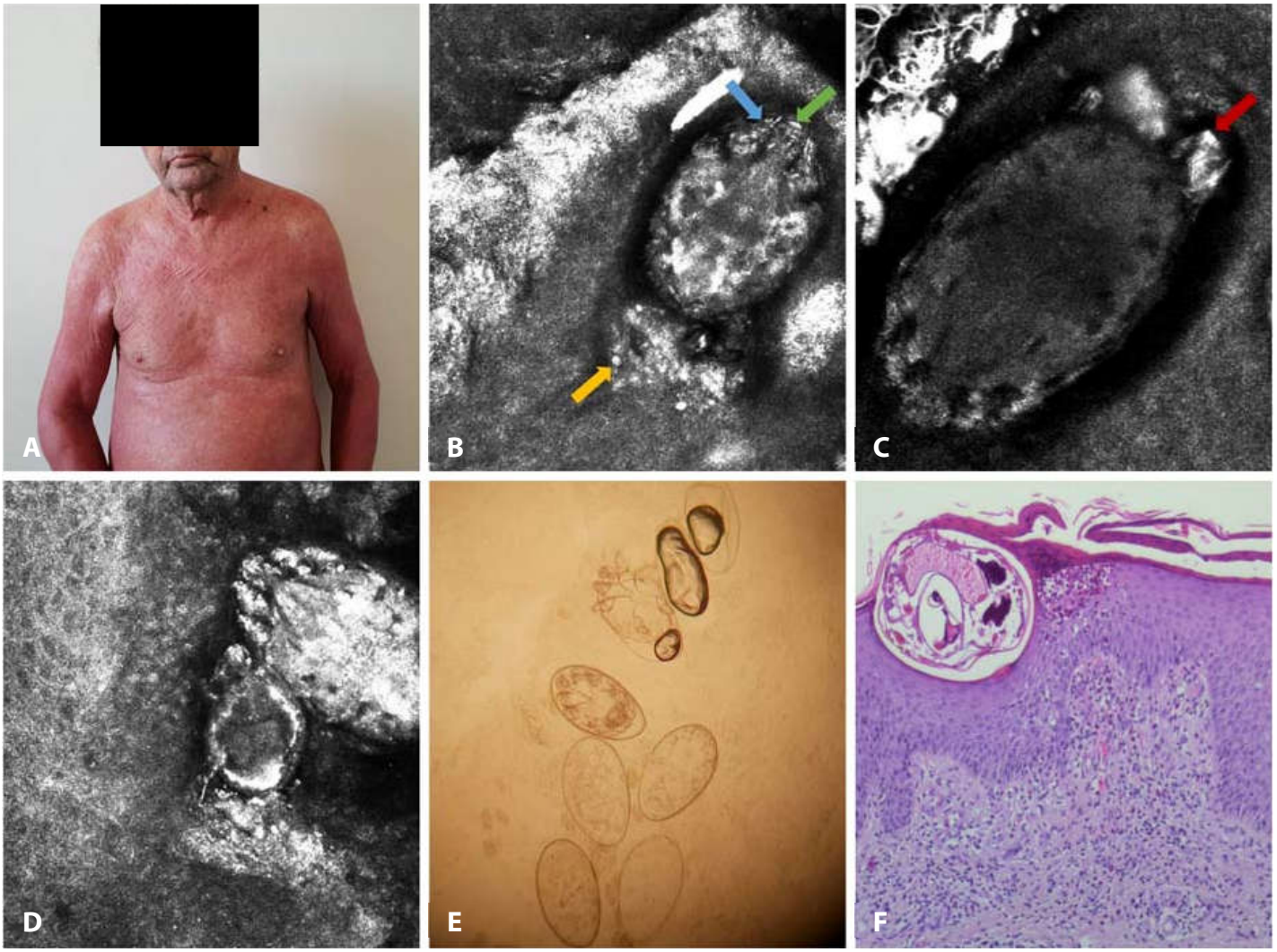

Figure 1. *Crusted scabies.* ***A)*** *Clinical picture showing erythroderma.* ***B)*** *RCM (basic image 0.5×0.5mm) at the level of the stratum corneum revealing a Sarcoptes scabiei mite with its droppings (yellow arrow). It is possible to observe Sarcoptes scabiei head (green arrow) and legs (blue arrow).* ***C)*** *RCM (basic image 0.5×0.5mm) also shows Sarcoptes scabiei eggs (red arrow).* ***D)*** *RCM (basic image 0.5×0.5mm) demonstrating a larva and an adult mite. Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.* ***E)*** *Microscopic examination (10×) of a skin scraping revealed multiple Sarcoptes scabiei mites.* ***F)*** *The histopathologic examination showed a mite within the epidermis and an eosinophilic inflammatory infiltrate. H&E,100×.*

To prevent the risk of infection during RCM examination with VivaScope 3000®, a disposable sterile transparent film should be applied to the camera tip or decontamination should be performed with a biocidal cleansing wipe. The traditional wide probe VivaScope 1500® is attached to the skin by using a disposable plastic interface.

This article provides an overview on the current applications of RCM for cutaneous infections and infestations in clinical practice. The review was performed using the PubMed database. Search terms in various combinations were "reflectance confocal microscopy," "skin," "hair," "nail," "infection," "parasitosis," "mycosis," "virus" and "bacteria." General characteristics of confocal images in selected infectious skin diseases are presented. All included RCM images were obtained from our department database and were acquired with Vivascope 1500®.

Parasitosis

Direct microscopic examination of skin scrapings was considered for many years, the gold standard for diagnosing scabies. However, this method has low sensitivity and may not be practical in the clinical

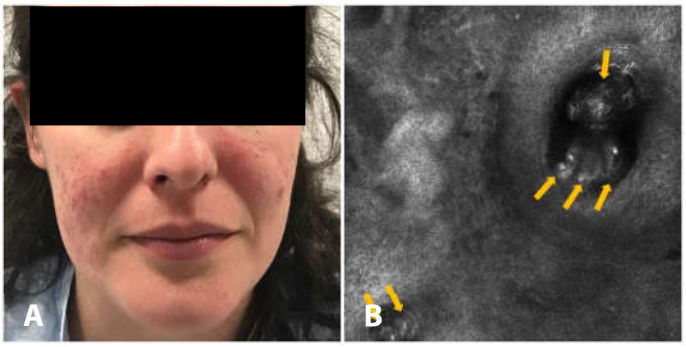

Figure 2. *Inflammatory rosacea.* ***A)*** *Clinical image shows central facial erythema and small erythematous papules.* ***B)*** *Under RCM (basic image 0.5×0.5mm), Demodex folliculorum mites (yellow arrows) appear as small round bodies with a hyper-reflective contours within hair follicles. Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.*

routine. Dermoscopy has a higher sensitivity, but the characteristic 'delta-wing jet' sign is sometimes difficult to identify.

Detection of *Sarcoptes scabiei* using RCM was first reported in 2005 [8]. Since then, several publications have described the usefulness of RCM for diagnostic confirmation of scabies [9-12]. On RCM, a burrow can be identified as a large, tortuous, hypo-refractive segment containing mites, eggs, or feces (scybala), (**Figure 1**). *Sarcoptes scabiei* appears as an inhomogeneously refractive ovoid body with short bright legs and a polygonal highly-refractive head [11]. Female mites are 400×300µm in size, whereas males are just over half this size. Moreover, the larvae can be distinguished from adults by their smaller size, fewer legs, and faster movement [2]. Scybala are easily detectable under RCM, as they appear as numerous, superficial and hyper-refractive oval bodies of 15µm in diameter; they indicate the presence of a mite. Eggs are hypo-refractive ovoid bodies of 200×100µm in size with a hyper-refractive thin wall [1].

Furthermore, RCM can be used to monitor the response to treatment by observing the mite's viability, eliminating unnecessarily repeated treatments. This technique allows recognition of dead mites by the absence of movements, cessation of vital functions (intestinal peristalsis, defecation), and hyper-reflecting appearance, with homogenization of internal structures and blurred edges [2]. Confocal microscopy is also useful for pathophysiological studies. It is able to locate and quantify the various forms of the mite in the epidermis and its activity in real time [13].

Demodex mites have been implicated in the pathogenesis of several inflammatory skin diseases, including rosacea, pityriasis folliculorum, perioral dermatitis, and blepharitis. The conventional tests used for their detection, such as superficial skin biopsy or skin scrapings, are indirect and semi-invasive and may cause discomfort to the patient. In contrast, RCM allows the detection of *Demodex folliculorum* in vivo and noninvasively within a few minutes [14]. The mite (**Figure 2**) presents as a roundish structure with a bright contour and a diameter of about 4-9µm, corresponding to the horizontal section of the parasite. It may also appear as a lengthy cone-shaped body corresponding to a sagittal section [15]. It is located upside-down within the follicular infundibulum, often in groups of three to five mites. *Demodex brevis*, which is usually found deep within the sebaceous glands, has not been identified by RCM so far [16].

Confocal microscopy permits the quantification of *Demodex folliculorum* with better precision as compared to standard skin surface biopsy [14]. Different studies using RCM have shown a significantly higher number of mites in patients with rosacea than in healthy subjects. Turgut et al. [16] found a mean number of mites per infested follicle of 3.17±0.96 in papulopustular rosacea, 1.90±1.14 in erythematotelangiectatic rosacea, and 0.74±1.02 in controls ($P<0.001$). Moreover, the density of *Demodex* mites in patients with rosacea under therapy can be monitored by RCM [17]. Ruini et al. [18] recently noticed a reduction in the brightness of residual mites and a loss of definition of their bright contours after topical application of ivermectin 10mg/g cream.

Confocal microscopic features of cutaneous leishmaniasis have also been described [19, 20]. This technique has the capacity to identify the cellular morphology of the immunologic response to these intracellular parasites, which are predominantly found in the papillary dermis. The most characteristic finding consists of bright interlacing fibers forming

roundish structures resembling bird's nests in the dermis (**Figure 3**). Within the bird's nest, follicles and granulomas are present as hyper-reflective oval structures, giving the distinctive picture of "eggs in a bird's nests" [20]. The granulomas are similar to hair follicles but smaller and disconnected from the skin surface. Other RCM aspects of cutaneous leishmaniasis include polymorphic inflammatory infiltrate, dilated longitudinal and curved vessels, and multinucleated giant cells located in the dermis. Amorphous material with moderate reflectivity and "brick-like" structures are seen within the superficial epidermis. Additionally, RCM is an efficient tool to corroborate the efficacy of treatment by demonstrating the disappearance of the characteristic structures, giving the appearance of "empty nests" (**Figure 4**).

Confocal microscopy has also been used to identify other cutaneous parasites [21-25]. *Pyemotes ventricosus*, an ectoparasite of arthropod larvae invading furniture, presents as an ovoid body of intermediate reflectance inside a cutaneous microvesicle [21]. Cutaneous *larva migrans* appears as a highly refractile oval or "S" shaped structure within a dark disruption in the normal honeycomb pattern of the epidermis (**Figure 5**), [22]. Furthermore, RCM allows one to study the anatomical details of bigger parasites [1, 23-25], such as ticks and lice, and to identify the skin hole caused by the bite of an insect.

Fungal infections

The clinical presentation of a fungal infection is not always clear and often poses diagnostic difficulties.

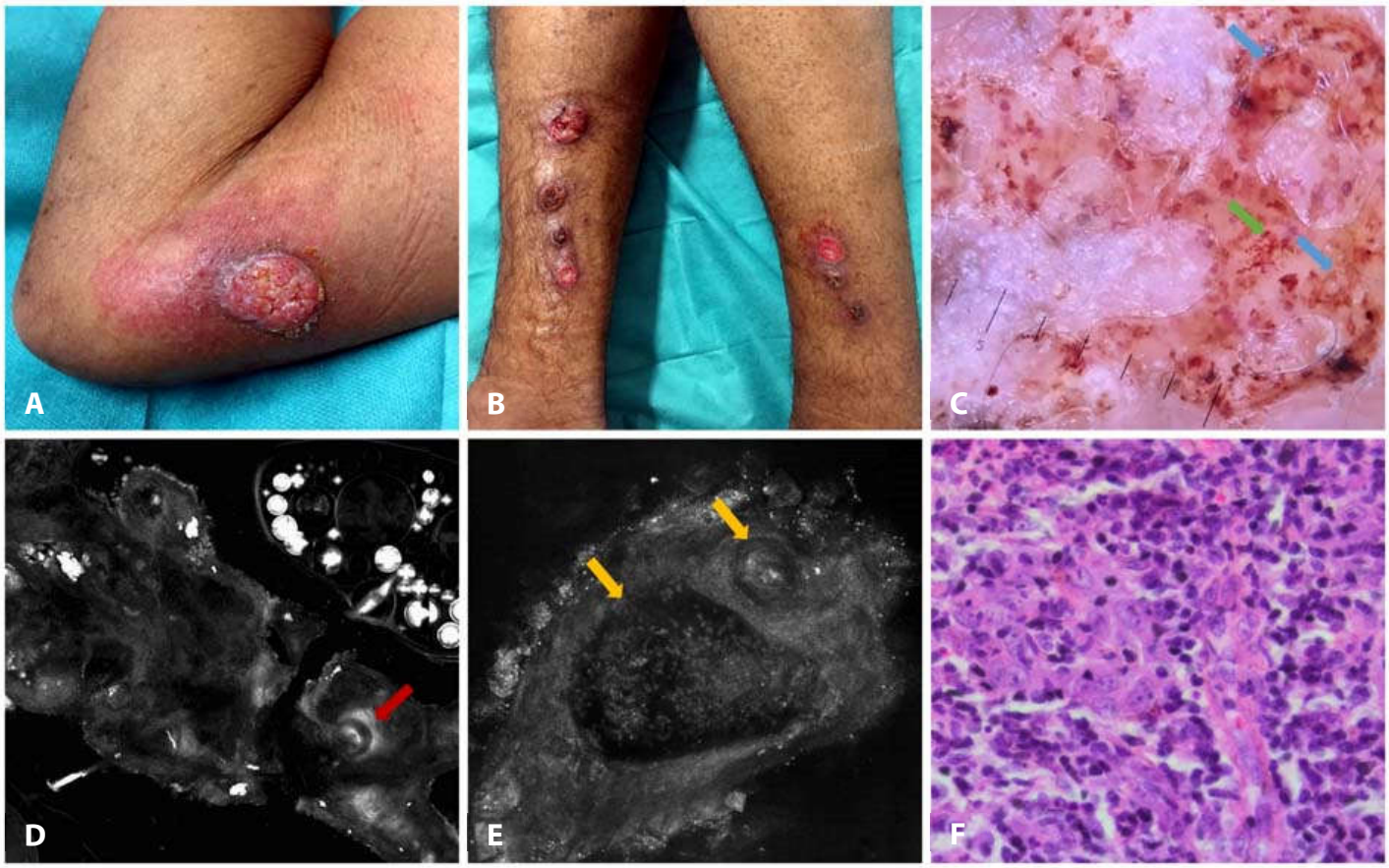

Figure 3. *Cutaneous leishmaniasis guyanensis. Clinical presentation: ulcerative plaques and tumors with erythematous-violaceous borders on the right arm **A)** and legs **B)**. A lymphocutaneous dissemination pattern is present on the legs. **C)** Dermoscopy reveals ulceration, yellow globules: "yellow drops" (blue arrows) and polymorphous vessels (green arrow). (D) RCM images of the papillary dermis [D), mosaic image 3x3mm; **E)**, basic image 0.5×0.5mm] show hyper-reflecting perifollicular fibers (red arrow) surrounding granulomas and multinucleated giant cells, forming the picture of "eggs in a bird's nest" (yellow arrows). Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA. **F)** Histopathologic examination showed basophilic cytoplasmic inclusion bodies in dermal histiocytes, corresponding to amastigotes. H&E,100×. Molecular techniques confirmed Leishmania guyanensis.*

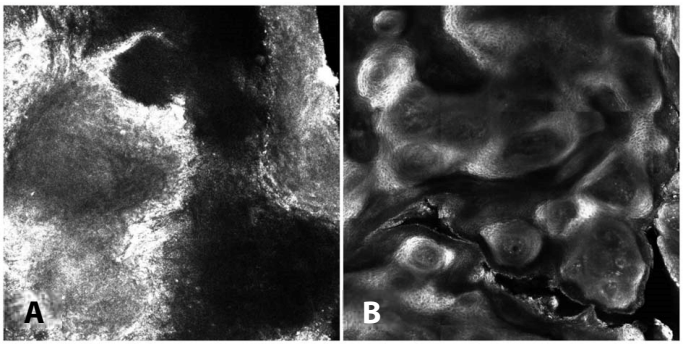

Figure 4. *Cutaneous leishmaniasis follow-up. After treatment, reassessment with confocal microscopy confirmed the disappearance of the previously identified structures, giving the appearance of "empty nests" A, basic image 0.5×0.5mm; B, mosaic image 2×2mm). Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.*

Conventional diagnostic tests such as direct light microscopic examination, fungal culture, and biopsy are to a variable extent invasive and time-consuming; incorrect results occur owing to sampling error. In contrast, in vivo RCM is a rapid, office-based, and noninvasive technique that allows confirmation of the diagnosis during dermatological consultation to allow prompt treatment. In addition, the entire surface of a skin lesion may be explored and the removal of scales for ex vivo analysis is not required.

The diagnosis of a fungal infection by RCM was first reported in 1994 in a patient with onychomycosis [26]. On RCM examination (**Figures 6, 7**), hyphae can be easily identified as bright, linear, branching and filamentous structures, whereas conidia appear as hyper-reflective small roundish bodies [27]. Hyphae

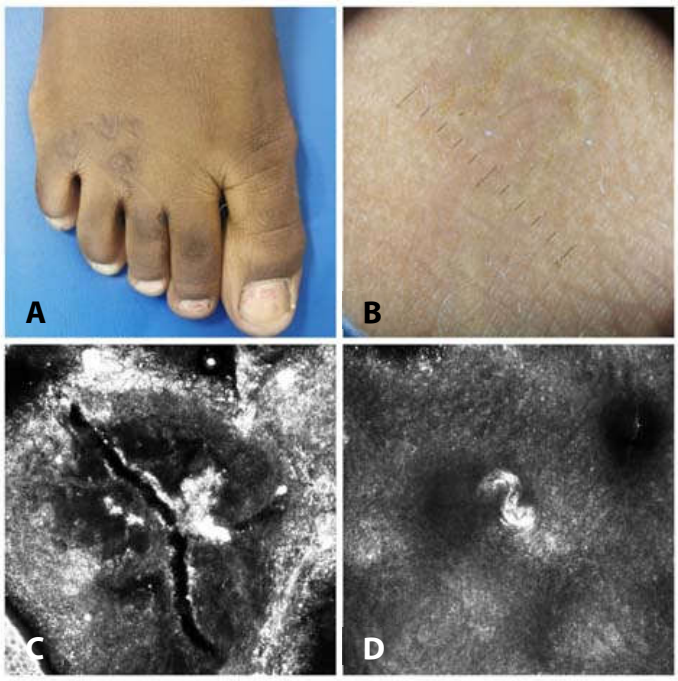

Figure 5. *Cutaneous larva migrans **A)** Serpiginous skin-colored eruption on dorsal right foot. **B)** Translucent brownish structures distributed linearly seen on dermoscopy. **C)** RCM imaging (basic image 0.5×0.5mm) showing a burrow, visible as a tortuous large empty space. **D)** RCM (basic image 0.5×0.5mm) showing a highly refractile "S" shaped structure, corresponding to larva migrans. Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.*

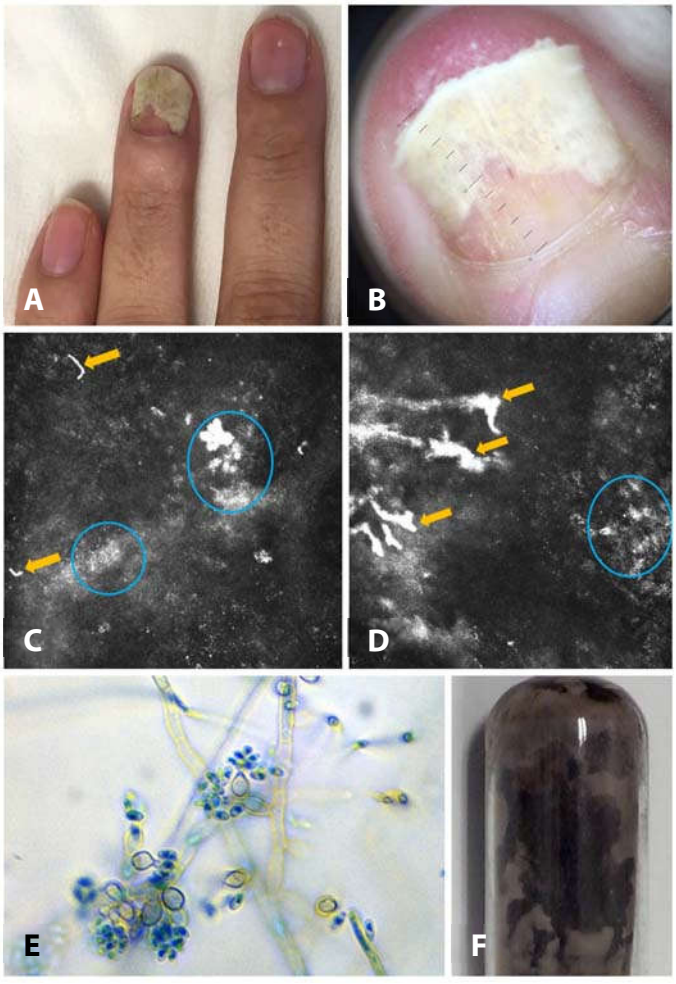

Figure 6. *Fusarium onychomycosis. **A)** Fingernail with white discoloration and onycholysis. **B)** Dermoscopy shows a white patch with jagged proximal edge. RCM images. **C, D)** Show bright filaments (yellow arrows) and conidia (blue circles), basic images 0.5×0.5mm. Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA. **E)** Microscopic examination with lactophenol blue staining revealed beanshaped macroconidia and some aseptate microconidia. **F)** Fusarium spp. on PDA culture medium.*

should be differentiated from the cell membranes of keratinocytes and from the normal structure of hair shafts.

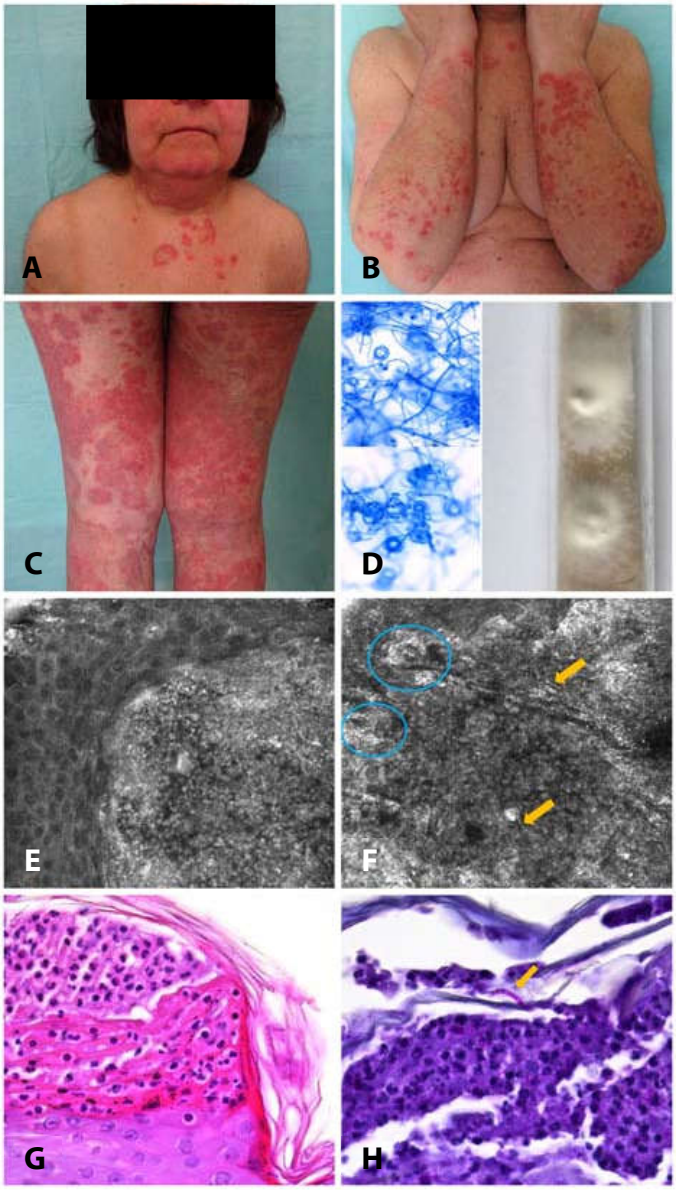

Figure 7. *Disseminated tinea incognito due to Trichophyton mentagrophytes var. mentagrophytes. Clinical presentation: extensive erythematous, sharply demarcated lesions with pustules on the face, trunk **A)**, arms **B)** and legs **C)**. **D)** Microscopic examination (lactophenol cotton blue stain) showed pear-shaped conidia along with spiral hyphae. **E)** The culture had a cream-colored, fine powdery surface and a white, velvety elevated border on PDA. **F)** RCM (basic image 0.5×0.5mm) reveals subcorneal pustules containing both neutrophils and acantholytic keratinocytes. **G)** RCM (basic image 0.5×0.5mm) at the stratum corneum showing bright filaments (yellow arrows) and conidia (blue circles). Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA. Correlated histopathological aspect of the subcorneal pustules. **H)** H&E, 400×, hypha (yellow arrow). **I)** Periodic acid-Schiff, 400×.*

Several studies have shown the applicability of RCM for diagnosis of dermatophytosis. Liansheng et al. [28] reported a sensitivity of 89.1% for tinea corporis, whereas Hui et al. [29] found a sensitivity of 63.6% for tinea manuum and pedis and 82.6% for tinea cruris. The accuracy of RCM in the diagnosis of onychomycosis was also investigated in two studies [30, 31], in which sensitivity ranged between 52.9% and 79.5% and specificity between 81% and 90.2%. The nail plate is mainly a homogeneous hypo-reflective medium, which allows a deep penetration of RCM (up to the nail bed in thin nails) and facilitates the visualization of fungi.

Another application of RCM is the diagnosis of hair dermatophytosis by the identification of ectothrix conidia and/or hyphae [32,33]. Dermatophytes inside the medulla of the hair shaft have not been identified.

The superiority of RCM over conventional microscopic examination for diagnosis of dermatophytosis has been demonstrated, but some results are mixed [34]. In a case-series of five patients with tinea incognito [35], RCM was found to be more sensitive than culture. In addition, this technique is particularly suitable for treatment monitoring.

In our experience it is still possible to identify *Malassezia* (**Figure 8**), which appears as clusters of roundish bright structures with tortuous hyper-reflective structures corresponding to thick and short septa analogues of the typical spaghetti and

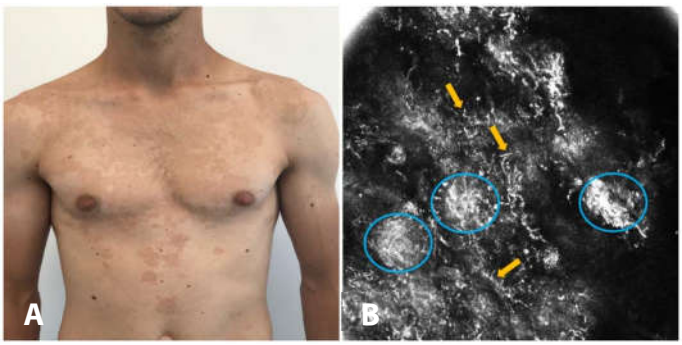

Figure 8. *Pityriasis versicolor. **A)** Well demarcated, thin, scaly and hyperpigmented plaques on the trunk. **B)** Under confocal microscopy (basic image 0.5×0.5mm), Malassezia appears as clusters of roundish bright structures with tortuous hyper-reflective structures analogues of the typical "spaghetti and meatballs" description. Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.*

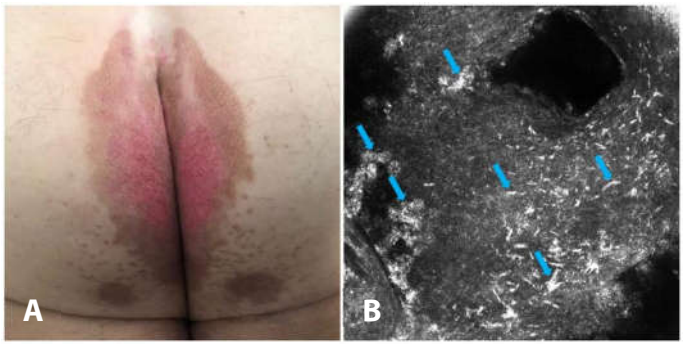

Figure 9. *Perianal candidiasis. **A)** Erythematous scaly plaques involving the perianal area. **B)** RCM (basic image 0.5×0.5mm) shows pseudofilaments and conidia (blue arrows). Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.*

meatballs description. *Candida*'s pseudofilaments and conidia have also been recognized by RCM in skin (Figure 9), oral mucosa, and nails [1]. Another important clinical application is the differentiation of tinea nigra from other melanocytic lesions [36-39].

Bacterial infections

The diagnosis of syphilis is often challenging and requires microscopic and laboratory tests. However, dark-field microscopy requires trained specialists and has limited sensitivity, whereas serology and immunohistochemistry require a waiting period for results. Recently, RCM was used for in vivo demonstration of *Treponema pallidu*m in cutaneous lesions of secondary syphilis (**Figure 10**), [4,5]. This technique disclosed rod shaped structures with regularly alternating hyper-reflective and non-reflective areas, corresponding to helix-shaped treponemes. Their sizes ranged from 4 to 16µm. Thus, confocal microscopy may be an effective and quick diagnostic tool for real-time imaging of *Treponema pallidum* in skin lesions. Nevertheless, its specificity and sensitivity remain to be evaluated.

This imaging technique could be also be used for an early diagnosis of impetigo. The optical sections demonstrate superficial subcorneal acantholysis and the presence of small bright inflammatory cells (**Figure 11**), [6].

One case of acrodermatitis chronica atrophicans [40], a late skin manifestation of Lyme disease, was also observed under RCM. A flattened surface with broadened skin folds, flattened dermo-epidermal junction with fewer papillae, and multiple small bright spots in the dermis were seen.

Viral infections

The cytopathic effect of herpes simplex virus, varicella-zoster virus, molluscipoxvirus, human papillomavirus and coxsackievirus have been identified using RCM. The ability of RCM in the recognition of cutaneous herpes infection (**Figure 12**) was first suggested in 2002 in a case of herpes simplex [41]. The authors reported the presence of multiple intraepidermal vesicles, appearing as dark spaces, containing large pleomorphic cells with dark cytoplasm, corresponding to ballooned keratinocytes and bright round structures corresponding to multinucleated giant cells

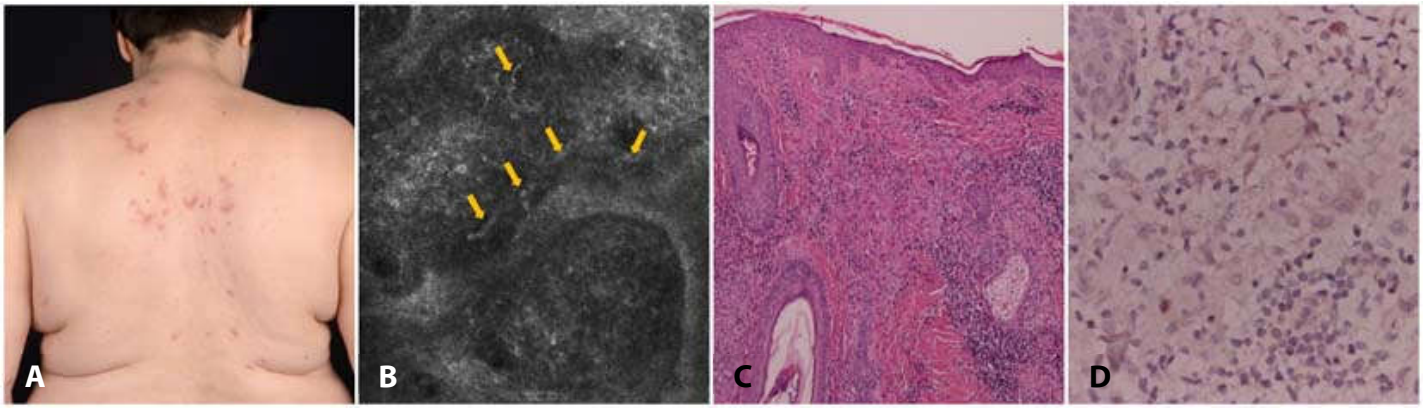

Figure 10. *Granulomatous secondary syphilis. **A)** Clinical presentation: erythematous scaly papulonodules on the trunk. **B)** RCM (basic image 0.5×0.5mm) demonstrated small elongated bright particles with a spiral shape (yellow arrows), corresponding to spirochetes, within multiple granulomatous foci. Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA. **C)** Histology revealed non-palisading epithelioid granulomas with numerous associated plasma cells. H&E, 100×. **D)** spirochetes were highlighted within the dermis by a Treponema pallidum immunostain.*

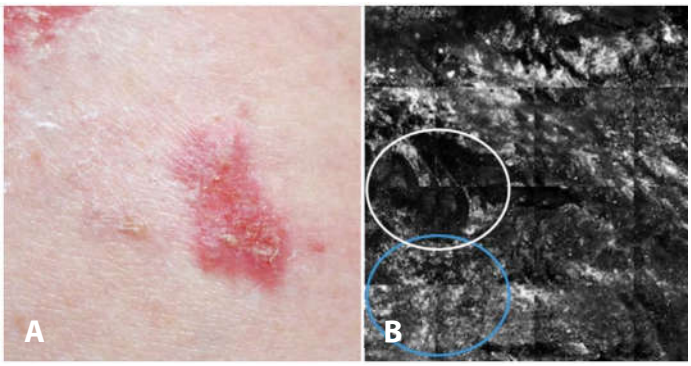

Figure 11. *Secondary bacterial infection (impetigo) of Hailey–Hailey disease.* ***A)*** *Erythematous plaque, erosions and yellowish crusts on the lumbar region of a patient with Hailey–Hailey disease.* ***B)*** *RCM (mosaic image 2×2mm) shows widened epidermal partial acantholysis (white circle) and infiltration of inflammatory cells (blue circle). Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.*

admixed with acantholytic keratinocytes and bright inflammatory cells. Subsequently, the same pattern was described in patients with varicella [42], herpes zoster [43] and Kaposi's varicelliform eruption [42]. Moreover, it was also reported that RCM can identify subtle epidermal changes in an early pre-vesicular stage when the clinical diagnosis is more difficult.

In molluscipoxvirus infections (**Figure 13**), RCM shows a round, well-circumscribed area consisting of hypo-refractive roundish lobules separated by fine septa and filled with hyper-refractive roundish bodies [44]. These structures correspond to the typical histopathologic features of lobulated, endophytic hyperplasia, and to the enlarged keratinocytes containing characteristic eosinophilic inclusion bodies (Henderson-Paterson bodies), respectively.

Confocal microscopy can also be used in the evaluation of flat and anogenital warts (**Figure 14**), [45-47]. The papillomatosis and enlarged capillary vessels are the identifying features. Moreover, at the level of the spinous-granulous layer, RCM may show koilocytosis, appearing as petal-like structures in flat warts and as large round cells in anogenital warts. These findings are difficult to identify in common warts related to the marked hyperkeratosis and acanthosis.

The diagnosis of all of these cutaneous infections is usually based on the typical clinical appearance, but

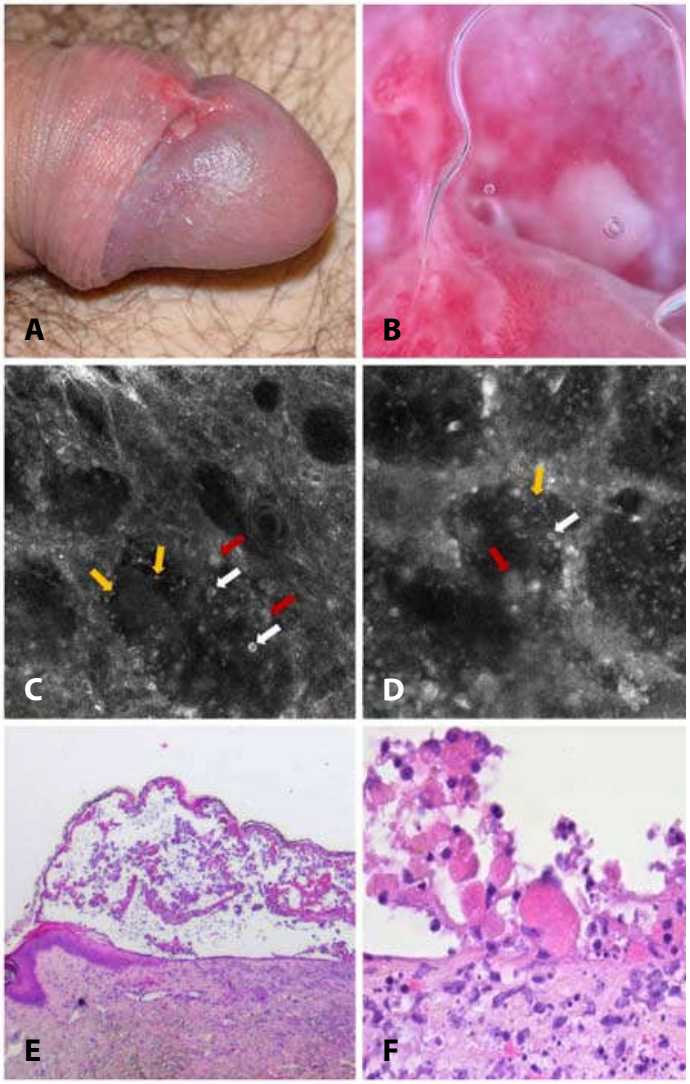

Figure 12. *Genital herpes.* ***A)*** *Grouped vesicles on an erythematous base on the glans penis.* ***B)*** *Dermoscopy shows a nonspecific pattern characterized by erythematous areas and dilated vessels. RCM examination,* ***C)*** *and* ***D)****, basic images reveal multiple intraepidermal vesicles containing bright acantholytic keratinocytes (yellow arrows), ballooned keratinocytes (white arrows), multinucleated giant cells (red arrows) and inflammatory cells, appearing as small bright particles, 0.5×0.5mm. Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA. Histopathology examination.* ***E)*** *H&E correlated well with the reflectance confocal microscopy features.* ***E)*** *100×;* ***F)*** *400×.*

some lesions may have an unusual morphology or can be located in uncommon body sites, particularly in the case of a pre-existing skin disease or in immunocompromised patients. In such cases, the diagnosis requires complementary investigations such as Tzanck cytodiagnosis, viral cultures, PCR, or histopathological examination.

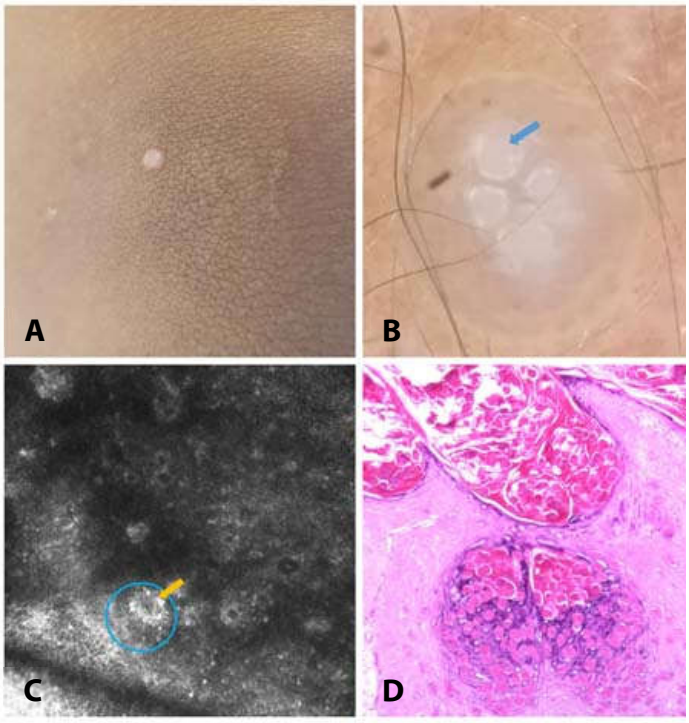

Figure 13. *Molluscum contagiosum.* ***A)*** *Clinical presentation: Dome-shaped, pearly papule with a central umbilication.* ***B)*** *Dermoscopy picture reveals central yellowish-white and polylobular amorphous structure (blue arrow).* ***C)*** *RCM (basic image 0.5×0.5mm) showing hypo-refractive roundish lobules (blue circle) containing hyper-refractive cells (yellow arrow). Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.* ***D)*** *Histopathology demonstrating well-define lobules containing enlarged keratinocytes with intracytoplasmic viral inclusions (Henderson-Paterson bodies).*

Confocal microscopy shows an excellent correlation with standard techniques. It allows rapid examination of multiple skin lesions in real time and is particularly suitable for children. This technique also permits very early identification of diagnostic features, allowing early treatment. In addition, RCM allows one to investigate the viral effects on a same location over time. However, its sensitivity and specificity need to be assessed and studies of comparison with standard methods should be performed.

Another application of RCM could be the early identification of vaginal intraepithelial neoplasia caused by human papillomavirus [1, 48]. Cinotti et al. also reported the identification of intraepidermal vesicles and ballooned cells with RCM in cases of hand, food and mouth disease induced by coxsackievirus infection [2].

Discussion

In vivo RCM has proved to be a non-invasive, painless, real-time imaging technique that can be used to identify cutaneous parasites not visible to the human eye (*Sarcoptes scabiei*, *Demodex folliculorum*, amastigote forms of *Leishmania*), body parts of bigger parasites and bacteria, fungi (yeasts, dermatophytes, molds), a few bacteria (*Treponema pallidum* spirochetes, impetigo), and cytopathic effects induced by some viruses (herpes simplex virus, varicella-zoster virus, molluscipoxvirus, and human papillomavirus).

Confocal microscopy allows for a detailed examination of different layers of the skin, with an accuracy that is comparable to that of histopathology. This technique can be efficiently performed during the patient's initial visit without requiring any further equipment. It may support the clinical diagnosis or indicate the need for further investigation, thereby reducing the need for time-consuming and invasive diagnostic procedures. Furthermore, the advent of handheld devices has enabled examination of multiple skin lesions within a few minutes and a better access to difficult anatomic sites such as the nose, eyelids, ears, folds, and mucosa [7]. These are particularly sensitive areas where non-invasive diagnostic techniques are of high interest.

In addition, RCM enables the evaluation of dynamic changes in the skin, which allows monitoring during treatment. Being non-invasive, RCM is also useful for study of pathophysiology of various skin conditions, allowing repeated evaluations of the same body site over time [2, 3].

There are some drawbacks to the use of RCM, including its limited depth of penetration (approx. 200-300µm). However, in most cases of infectious skin diseases, the main alterations are found in the epidermis and upper dermis where the resolution of confocal microscopy is nearly similar to that of histopathology [2]. The high cost of the equipment

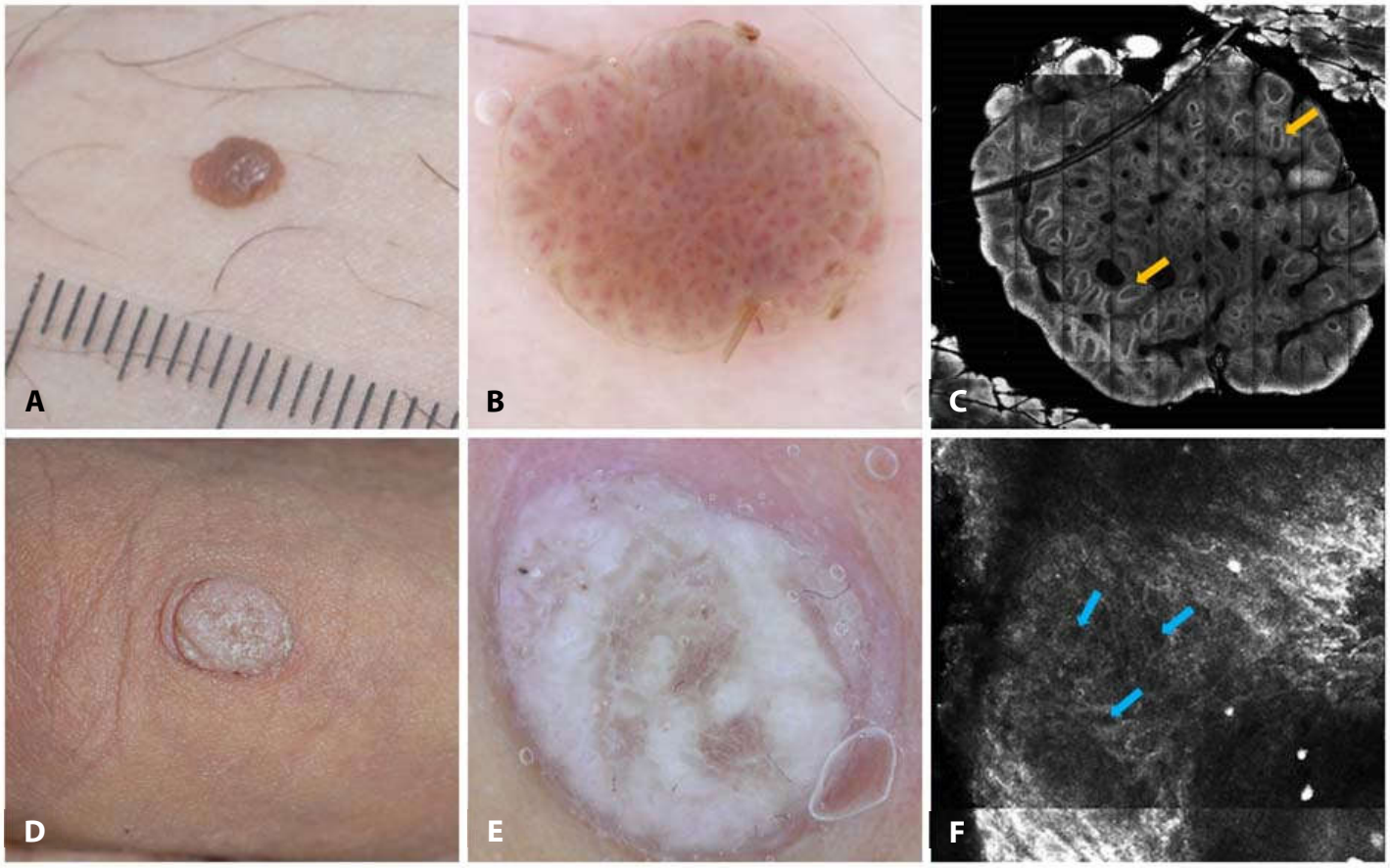

Figure 14. *Genital warts.* ***A)*** *Clinical presentation: dome-shaped, verrucous and brown papule on the suprapubic region.* ***B)*** *Dermatoscopic pattern with flattened and rounded structures and glomerular vessels.* ***C)*** *RCM (mosaic image 5×5mm) showing papillomatosis and dilated vessels (yellow arrow).* ***D)*** *Hyperkeratotic dome-shaped papule on the penile shaft.* ***E)*** *Dermoscopy reveals keratosis and rounded closely aggregated knoblike projections.* ***F)*** *RCM (mosaic image 0.6×0.6mm) at the spinous layer demonstrates a honeycomb pattern, with some larger cells that correspond to koilocytosis. Reflectance confocal microscopy: VivaScope 1500®, Caliber: imaging and diagnostics, Rochester, NY, USA.*

and need for trained physicians to read the images are other limiting factors. Nonetheless, in dermatological centers where RCM is routinely applied for the detection of skin tumors, cutaneous infestations and infections may be an additional application. With the growing number of indications and advances in technology, RCM will certainly be widely available. Further studies are needed to compare the RCM performance with that of conventional diagnostic techniques and to determine other possible patterns in larger number of infections.

Conclusion

This review highlights the suitability of RCM in the diagnosis of skin infestations and infections. Confocal microscopy allows real-time identification of cutaneous parasites, fungi, bacteria, and viral cytopathic effects not visible to the naked eye. Treatment monitoring can also be performed with this technique. Further studies, including larger case series, will better define RCM features of cutaneous infections and will help create guidelines for implementation of this technique in clinical practice.

Potential conflicts of interest

The authors declare no conflicts of interests.

References

1. Hoogedoorn L, Peppelman M, van de Kerkhof PC, van Erp PE, Gerritsen MJ. The value of in vivo reflectance confocal microscopy in the diagnosis and monitoring of inflammatory and infectious skin diseases: a systematic review. *Br J Dermatol*. 2015;172(5):1222-48. [PMID: 25355622].
2. Cinotti E, Perrot JL, Labeille B, Cambazard F. Reflectance confocal microscopy for cutaneous infections and infestations. *J Eur Acad Dermatol Venereol*. 2016;30:754–63. [PMID: 26387660].
3. Cinotti E, Labeille B, Cambazard F, Perrot JL. Reflectance confocal microscopy in infectious diseases. *G Ital Dermatol Venereol*. 2015;150:575–83. [PMID: 26129682].
4. Venturini M, Sala R, Semenza D, et al. Reflectance confocal microscopy for the in vivo detection of Treponema pallidum in skin lesions of secondary syphilis. *J Am Acad Dermatol*. 2009;60(4):639-42. [PMID: 19293012].
5. Leclercq A, Cinotti E, Labeille B et al. Apport de la microscopie confocale parréflectance dans le diagnostic de syphilidesano-vulvaires, premier cas relaté. *Ann Dermatol Venereol*. 2016;143(11):687-690. [PMID: 27567281].
6. Debarbieux S, Depaepe L, Poulalhon N, et al. Reflectance confocal microscopy characteristics of eight cases of pustular eruptions and histopathological correlations. *Skin Res Technol*. 2013;19(1):e444-52. [PMID: 22934633].
7. Cinotti E, Labeille B, Cambazard F, Perrot JL. Confocal Microscopy for Special Sites and Special Uses. *Dermatol Clin*. 2016;34(4):477-485. [PMID: 27692453].
8. Longo C, Bassoli S, Monari P, Seidenari S, Pellacani G. Reflectance-Mode Confocal Microscopy for the In Vivo Detection of Sarcoptes scabiei. *Arch Dermatol*. 2005;141(10):1336. [PMID: 16230583].
9. Lacarrubba F, Verzì AE, Micali G. Detailed Analysis of In Vivo Reflectance Confocal Microscopy for Sarcoptes scabiei hominis. *Am J Med Sci*. 2015;350(5):414. [PMID: 25211585].
10. Gürel MS, Turgut Erdemir AV, Tekin B. A Case Report of Real-Time in vivo Demonstration of Sarcoptes scabiei. *Turkiye Parazitol Derg*. 2017;41(4):229-232. [PMID: 29318996].
11. Francisco G, Eilers S, Haroon A et al. No wonder it itches: quick bedside visualization of a scabies infestation using reflectance confocal microscopy. *J Cutan Pathol*. 2018;45(12):877-879. [PMID: 30419160].
12. Levi A, Mumcuoglu KY, Ingber A, Enk CD. Detection of living Sarcoptes scabiei larvae by reflectance mode confocal microscopy in the skin of a patient with crusted scabies. *J Biomed Opt*. 2012;17(6):060503. [PMID: 22734726].
13. Cinotti E, Perrot JL, Labeille B et al. Reflectance confocal microscopy for quantification of Sarcoptes scabiei in Norwegian scabies. *J Eur Acad Dermatol Venereol*. 2013;27(2):e176-8. [PMID: 22621304].
14. Sattler EC, Maier T, Hoffmann VS, et al. Noninvasive in vivo detection and quantification of Demodex mites by confocal laser scanning microscopy. *Br J Dermatol*. 2012;167:1042-7. [PMID: 22716072].
15. Harmelin Y, Le Duff F, Passeron T, Lacour JP, Bahadoran P. The value of reflectance confocal microscopy in detection of Demodex mites. *Ann Dermatol Venereol*. 2017;144(6-7):459-461. [PMID: 28347561].
16. Turgut Erdemir A, Gurel MS, Koku Aksu AE, et al. Demodex mites in acne rosacea: reflectance confocal microscopic study. *Australas J Dermatol*. 2017;58(2):e26-e30. [PMID: 26969834].
17. Sattler EC, Hoffmann VS, Ruzicka T, Braunmuhl TV, Berking C. Reflectance confocal microscopy for monitoring the density of Demodex mites in patients with rosacea before and after treatment. *Br J Dermatol*. 2015;173:69-75. [PMID: 25801631].
18. Ruini C, Sattler E, Hartmann D, et al. Monitoring structural changes in Demodex mites under topical Ivermectin in rosacea by means of reflectance confocal microscopy: a case series. *J Eur Acad Dermatol Venereol*. 2017;31(6):e299-e301. [PMID: 27976437].
19. Buljan M, Zalaudek I, Massone C, et al. Dermoscopy and reflectance confocal microscopy in cutaneous leishmaniasis on the face. *Australas J Dermatol*. 2016;57(4):316-318. [PMID: 26424528].
20. Alarcon I, Carrera C, Puig S, Malvehy J. In vivo confocal microscopy features of cutaneous leishmaniasis. *Dermatology*. 2014;228(2):121-4. [PMID: 24503465].
21. Del Giudice P, Blanc-Amrane V, Bahadoran P et al. Pyemotes ventricosus dermatitis, southeastern France. *Emerg Infect Dis*. 2008;14(11):1759-61. [PMID: 18976564].
22. Purdy KS, Langley RG, Webb AN, Walsh N, Haldane D. Cutaneous larva migrans. *Lancet*. 2011;377(9781):1948. [PMID: 21601273].
23. Aksu AE, Erdemir VA, Gurel MS, Sarikaya E, Ozkur E. In vivo evaluation of Phthirus pubis with reflectance confocal microscopy. *Parasitol Res*. 2015;114(9):3559-61. [PMID: 26099238].
24. Cinotti E, Labeille B, Cambazard F, et al. Unusual reflectance confocal microscopy findings during the examination of a perianal nevus: pinworms. *J Eur Acad Dermatol Venereol*. 2016;30(10):e86-e87. [PMID: 26374669].
25. Cinotti E, Labeille B, Bernigaud C et al. Dermoscopy and confocal microscopy for in vivo detection and characterization of Dermanyssus gallinae mite. *J Am Acad Dermatol*. 2015;73(1):e15-6. [PMID: 26089062].
26. Piérard GE, Arrese JE, Pierre S et al. Microscopic diagnosis of onychomycoses. *Ann Dermatol Venereol*. 1994;121(1):25-9. [PMID: 8092723].
27. Cinotti E, Perrot JL, Labeille B et al. Tinea corporis diagnosed by reflectance confocal microscopy. *Ann Dermatol Venereol*. 2014; 141(2):150-2. [PMID: 24507212].
28. Liansheng Z, Xin J, Cheng Q, Zhiping W, Yanqun L. Diagnostic applicability of confocal laser scanning microscopy in tinea corporis. *Int J Dermatol*. 2013;52(10):1281-2. [PMID: 24073912].
29. Hui D, Xue-cheng S, Ai-e X. Evaluation of reflectance confocal microscopy in dermatophytosis. *Mycoses*. 2013;56(2):130 - 133. [PMID: 22963376].
30. Pharaon M, Gari-Toussaint M, Khemis A et al. Diagnosis and treatment monitoring of toenail onychomycosis by reflectance confocal microscopy: Prospective cohort study in 58 patients. *J Am Acad Dermatol*. 2014;71(1):56-61. [PMID: 24786418].
31. Rothmund G, Sattler EC, Kaestle R et al. Confocal laser scanning microscopy as a new valuable tool in the diagnosis of onychomycosis – comparison of six diagnostic methods. *Mycoses*. 2013;56(1):47-55. [PMID: 22524550].
32. Cinotti E, Perrot JL, Labeille B, et al. Hair dermatophytosis diagnosed by reflectance confocal microscopy: six cases. *J Eur Acad Dermatol Venereol*. 2015; 29(11):2257-9. [PMID: 24854883].
33. Veasey JV, Meneses OMS, da Silva FO. Reflectance confocal microscopy of tinea capitis: comparing images with results of dermoscopy and mycological examinations. *Int J Dermatol*. 2019;58(7):849-851. [PMID: 30229878].
34. Navarrete-Dechent C, Bajaj S, Marghoob AA, Marchetti MA. Rapid diagnosis of tinea incognito using handheld reflectance confocal microscopy: a paradigm shift in dermatology? *Mycoses*. 2015;58(6):383-6. [PMID: 25919861].

35. Turan E, Erdemir AT, Gurel MS, Yurt N. A new diagnostic technique for tinea incognito: In vivo reflectance confocal microscopy. Report of five cases. *Skin Res Technol*. 2013;19(1):e103-7. [PMID: 22676444].
36. Mateus R, Murzaku EC, Rao BK. Atypical pigmented acral lesion proven to be fungal using reflectance confocal microscopy. *J Am Acad Dermatol*. 2014;70(4):e77-e79. [PMID: 24629366].
37. Veasey JV, Avila RB, Ferreira MAMO, Lazzarini R. Reflectance confocal microscopy of tinea nigra: comparing images with dermoscopy and mycological examination results. *An Bras Dermatol*. 2017;92(4):568-569. [PMID: 28954116].
38. Cheng HM, Thng ST, Tan WP, Chuah SY. Handheld reflectance confocal microscopy: A useful tool to aid diagnosis of acral pigmented lesions. *J Dermatol*. 2016;43(8):980-1. [PMID: 27075596].
39. Uva L, Leal-Filipe P, Soares-de-Almeida L, et al. Reflectance confocal microscopy for the diagnosis of tinea nigra. *Clin Exp Dermatol*. 2018;43(3):332-334. [PMID: 29283186].
40. Gülseren D, Cerroni L, Hofmann-Wellenhof R, Arzberger E. Correlation of Reflectance Confocal Microscopy and Dermatopathology Findings in a Case of Acrodermatitis Chronica Atrophicans. *Am J Dermatopathol*. 2018;40(5):367-370. [PMID: 29251638].
41. Goldgeier M, Alessi C, Muhlbauer JE. Immediate noninvasive diagnosis of herpesvirus by confocal scanning laser microscopy. *J Am Acad Dermatol*. 2002;46:783-785. [PMID: 12004325].
42. Lacarrubba F, Verzì AE, Pippione M, Micali G. Reflectance confocal microscopy in the diagnosis of vesicobullous disorders: case series with pathologic and cytologic correlation and literature review. *Skin Res Technol*. 2016;22(4):479-486. [PMID: 27259839].
43. Lacarrubba F, Verzì AE, Musumeci ML, Micali G. Early diagnosis of herpes zoster by handheld reflectance confocal microscopy. *J Am Acad Dermatol*. 2015; 73:e201-3. [PMID: 26568349].
44. Lacarrubba F, Verzì AE, Ardigò M, Micali G. Handheld reflectance confocal microscopy for the diagnosis of molluscum contagiosum: histopathology and dermoscopy correlation. *Australas J Dermatol*. 2017;58(3):e123–5. [PMID: 27282954].
45. Lacarrubba F, Ardigò M, Di Stefani A, Verzì AE, Micali G. Dermatoscopy and Reflectance Confocal Microscopy Correlations in Nonmelanocytic Disorders. *Dermatol Clin*. 2018;36(4):487-501. [PMID: 30201157].
46. Veasey JV, Framil VMS, Nadal SR, Marta AC, Lellis RF. Genital warts: comparing clinical findings to dermatoscopic aspects, in vivo reflectance confocal features and histopathologic examination. *An Bras Dermatol*. 2013;89(1):137-40. [PMID: 24626658].
47. Hui D, Hong-Yan J, Ai-E X. Evaluation of verruca plana by in vivo reflectance confocal microscopy and dermoscopy. *Skin Res Technol*. 2017;23(3):437-440. [PMID: 28185315].
48. Blumetti TP, Scope A, de Macedo MP et al. Dermoscopic and Reflectance Confocal Microscopy Findings in Extra-genital HPV16-associated Pigmented Squamous Cell Carcinoma in situ. *Acta Derm Venereol*. 2016;23;96(6):836-7. [PMID: 26976508].