published by:	AWMF online Das Portal der wissenschaftlichen Medizin

AWMF registry no. (German Association of Scientific Medical Professional Societies):	**013/076**	**Class:**	**S1**

Guideline: Confocal Laser Microscopy in Dermatology

Update of the guideline from version dated: 01.07.2011

Date of completion: 28.07.2017

Development stage: S1

Responsible expert association(s): German Dermatological Society (DDG)

Type of consensus building: Consensus-building within the representative group of experts took place by email and in personal meetings with repeated votes by the experts involved.

Validity: 5 Years

This guideline was reviewed by the 2+2 Commission of the Commission for Quality Assurance in Dermatology and adopted by the German Dermatological Society.

Julia Welzel, Martina Ulrich, Susanne Lange-Asschenfeldt, Wilhelm Stolz, Elke Sattler

Target and addressees: Dermatologists who want to gain an overview of the possible uses of confocal laser microscopy because they are considering using this technique for diagnostics. Dermatologists who are already working with confocal laser microscopy and who would like to receive instructions for use and an overview of the indications and limitations of the method.

1. Introduction

Confocal laser microscopy (CLM) is a non-invasive method for high-resolution tissue diagnostics. While conventional microscopes use transmitted-light technology to illuminate thin tissue layers from below, confocal laser microscopy, designed for dermatology, uses incident-light technology. In each case, laser light of a selected wavelength is used to illuminate the section of skin to be examined. The laser beam is first focused on a plane within the skin, where the light is reflected at boundary layers with a high refractive index and is then directed to a detector. An upstream pinhole allows only signals from the previously defined horizontal plane to be used for imaging. Structures with high reflectance in the skin are mainly keratin, melanin and collagen, or boundary layers with a very different refractive index. The method is thus mainly suitable for

diagnosis of melanocytic and epithelial skin tumours.[88] While this procedure on the one hand allows changes near the surface to be depicted in high resolution, using a microscopic resolution of 1 to 3 µm, in horizontal sections, it also limits the penetration depth into the skin.

Confocal laser microscopy thus allows new possibilities for diagnostics and progress monitoring within dermatology. This applies, in particular, to the investigation of dynamic changes. However, the technique can also be used ex vivo on freshly excised tissue in the sense of frozen section diagnostics. The latter is particularly interesting for the field of microscopically controlled surgery for skin tumours.

When using monochromatic laser light and suitable filters, fluorescence can be used for imaging in addition to reflectance. To do this, the skin must be stained with fluorescent dyes, either externally or via intradermal injection.

2. Appliances

For confocal laser microscopy, devices are used which have one or more lasers as a light source, which can be used both for in-vivo and ex-vivo examination of the skin. The laser energy at tissue level is less than 30 mW, so there is no danger to the tissue being examined or to the human eye (laser class I).

There is currently only one provider of confocal laser microscopes for skin diagnostics, the company Mavig GmbH (Munich, Germany).

The following devices are in clinical use for in-vivo diagnostics: the VivaScope® 1500, the VivaScope® 1500 Multilaser, and the VivaScope®3000.[49] The equipment is completed by a video-dermatoscopic unit (VivaCam®, Visiomed AG, Bielefeld) which needs to be connected. The VivaScope® 1500 is the standard device for in-vivo diagnostics in reflectance mode. The VivaScope® 1500 Multilaser incorporates three lasers of different wavelengths for examinations in reflectance and fluorescence modes. The VivaScope® 3000 is a mobile handheld device for diagnosing anatomically difficult to reach skin alterations.

Ex-vivo confocal laser microscopy enables the examination of tissue sections without staining. The VivaScope® 2500 is a multi-laser KLM with the wavelengths of 830 nm for reflectance diagnostics and 488 nm and 658 nm for fluorescence diagnostics. The device design differs from the in-vivo devices, because, here, the fresh tissue is placed on a measuring table and illuminated from below. To place the tissue as flat as possible onto the measuring table, it needs to be fixed by means of slides or embedding media. An overview of the devices and configurations is given in Table 1.

Tab. 1:

Name	**Laser**	**Image size**	**Imaging**	**Area of application**
VivaScope® 1500	830 nm	Single image 500 µm x 500 µm, mosaic up to 8 mm x 8 mm	Reflectance	In-vivo diagnostics
VivaScope® 1500 Multiwave	488 nm, 658 nm, 785 nm	Single image 500 µm x 500 µm, mosaic up to 8 mm x 8 mm	Reflectance and fluorescence	In vivo, experimental clinical questions
VivaScope® 3000	830 nm	Single image 1000 µm x 1000 µm, no mosaic function	Reflectance	In vivo, for hard-to-reach areas, for fast, flexible measurements
VivaScope® 2500	488 nm, 658 nm, 830 nm,	750 µm x 750 µm, mosaic up to 20 mm x 20 mm	Reflectance and fluorescence	Ex vivo, for micrographic surgery, margin control

Table 1: Device Configurations for Confocal Laser Microscopy

3. Indications

In dermatology, confocal laser microscopy is suitable for non-invasive diagnosis of near-surface skin alterations. In the field of skin tumours, it is of particular interest for assessing melanocytic lesions with regard to malignancy, in order to be able to detect melanoma early, and, on the other hand, avoid unnecessary excisions of benign nevi. Non-invasive early detection is also important in the case of epithelial skin tumours, along with follow-up and therapeutic monitoring, especially if non-surgical, topical therapies are used where no histologies are taken.
Even superficial inflammatory skin diseases can be examined using confocal laser microscopy. Here, it is not so much the diagnostics that are in the foreground, but the progress monitoring and quantification of therapeutic effects.
There are limitations to this methodology when there are deeper tumour components or inflammation, which do not get represented due to the low optical penetration depth of this technique. The horizontal representation of the tissue layers requires a change in thinking when it comes to the interpretation of images, even for a histologist experienced in deep sections.
Ex-vivo confocal laser microscopy is suitable for frozen section diagnostics and microscopic resection margin control. A major advantage over conventional histology is that no time-consuming tissue preparation is required, so the results are available within a few minutes.

4. In-vivo confocal laser microscopy

4.1 Examination technique

Since movement artefacts must be minimised as much as possible due to the method's high resolution, and as the measurement takes a few minutes, the examination should be performed with the patient in a relaxed position, lying down. First, a magnetic ring with a transparent window is glued to the

lesion being examined, which is wetted with a drop of immersion oil in advance to minimise the reflectance of the skin's surface. Then a dermatoscopic image is taken using the VivaCam®. The large measuring head is fixed to the magnetic ring, which should have already been filled with ultrasound gel for coupling the lens, and the skin's surface is focused on using the lens system. This is followed by standardised images of the lesion on at least three planes (so-called mosaics or blocks) at the level of the upper dermis, the dermoepidermal junction and the upper dermis in the x-y direction. The area of the planes can be freely selected up to a size of 8 mm x 8 mm. Consecutive scans, sized 500 µm × 500 µm, are then driven in narrow sections into the skin's depth in the z-direction (so-called stacks) on at least three selected individual images from the centre of the lesion. It is recommended to include the adjacent healthy skin for comparison. The exact position of the stacks and the planes is displayed in the dermatoscopy image, where you can also navigate. The measurement is carried out in real time, so that you see, for example, the flow of blood cells in the blood vessels. A small video sequence can also be recorded to document dynamic processes. The entire recording procedure takes about eight to ten minutes. The measuring head is then decoupled, the magnetic ring removed, the skin cleaned of oil, and the images evaluated.

The VivaScope® 3000 is a flexible handpiece with which you can record only single images sized1000 µm x 1000 µm, and no mosaics. However, recording a stack down to a depth of 200 µm is also possible. It is suitable for mobile recording of lesions in skin folds and on curved surfaces. The small, light handpiece is fixed using only the hands. The measurement takes only a few seconds. An overview image of an entire lesion is not possible here, and so assignment of the individual images is more difficult.

4.2 Depiction of healthy skin

During the examination of healthy areas of the skin, the stratum corneum appears at the top of the image.[50] The polygonal corneocytes, without nuclei, form a cohesive, highly refractile cell cluster with the furrow pattern, plication and creases typical of normal skin, which appear as dark lines between the aggregated

corneocytes. Individual corneocytes are depicted in CLM with a size of 20 to 30µm.[25,50]

Depicted along with them is the stratum granulosum, consisting of 2 to 4 cell layers with a single cell size of between 20 and 25 µm. The cell nuclei appear centrally as dark, oval-round structures, surrounded by a narrow ring of bright cytoplasm with a granular appearance. The next layer is the stratum spinosum, with polygonal cells with a size of 15 to 20 µm. These are arranged in a characteristic honeycomb pattern, which in some places allows the first pigmented basal cells to be made out above the papillary tips, which can result in a cobblestone-like pattern. The basal cell layer itself consists of cells with differing levels of refractility, corresponding to the varying melanin content of the Fitzpatrick phototypes. The melanin content correlates with the corresponding reflectivity and thus with the image brightness.[8,72,120] The cell size is between 10 and 12 µm. In the dermo-epidermal junction, the basal cells form bright rings around the central, dark papillae. The flow of blood in the superficial capillaries can usually be visualised within the papillary tips.

Below the junction zone are depicted the reticular bundles of dermal connective tissue, where topographical and age-dependent differences are seen in their arrangement, density and reflectivity.

Skin appendages such as hair follicles, sebaceous glands and excretory ducts of eccrine glands can also be visualised using CLM. Here, excretory eccrine ducts appear as a spiral-like light structures in the epidermis; sebaceous glands appear as round, coil-like formations, with a hair in the middle, which is depicted as a linear, brightly reflective structure and has a characteristic stratification.

Further topographical differences exist between hairy skin and the hairless skin of the palms and soles of the feet. For one thing, the latter has a much thicker horny layer, which can be measured with the help of a micrometer and shows the regular distribution of pore-like openings of the eccrine glands, which appear dark in CLM.[50]

4.3 Tumours

Confocal laser microscopy has already been used to study neoplastic skin alternations during the first stages of development, with a focus on the study of malignant melanoma and its differentiation from benign melanocytic proliferation. Another focus was on the study of light skin cancer, defining the CLM criteria of actinic keratoses, of basal cell carcinoma, and of related diseases such as Bowen's disease. [2,4,5,6,24,27,29,32,33,40,44,47,48,55,60,65,70,74,76-81,86,91,96,108-110,118,119.]

4.3.1 Malignant melanoma

Malignant melanoma was already the subject of systematic studies in confocal laser microscopy early on, since melanocytic lesions can be visualised very well due to the strong endogenous contrast of melanin. In recent years, the clinical applicability of CLM for melanoma diagnostics has been demonstrated in numerous studies. Defined morphological imaging characteristics of melanomas and benign melanocytic lesions have been developed in this context. In this connection, elimination of the normal epidermal architecture, a lack of delineation of the papillae (so-called non-edged papillae), irregular nests of atypical melanocytes, and the presence of large, highly refractile cells with prominent nuclei in higher epidermis layers are considered the most important criteria for melanoma.

Studies show that the use of CLM leads to an improvement in the specificity of melanoma diagnostics. In addition, CLM can significantly reduce the so-called "number needed to excise" (NNE), i.e., the number of excisions of benign nevi in order to find a melanoma.[5,7,30,32,44,60,76-83,85,98,100,101]

4.3.2 Actinic keratosis

In CLM, actinic keratosis is characterised by a loss of the normal honeycomb structure, with atypia and pleomorphism of the epidermal keratinocytes, parakeratosis, detached corneocytes in the stratum corneum, and solar elastosis,

as well as blood vessel dilation. Horizontal overview maps often show overlying compact hyperkeratosis, which can make the visualisation of deeper-lying structures considerably more difficult in some cases. This is also the reason for KLM's limitation in the diagnosis of invasive squamous cell carcinoma, as severe hyperkeratosis often limits the evaluation of the lesions. [4,6,47,48,69,84,87,99,106,108-111,113-115,121]

4.3.3 Basal cell carcinoma

Basal cell carcinomas show characteristic alterations in CLM, with early studies describing the following five main criteria: elongated, monomorphic nuclei, polarisation of these cells along one axis, marked inflammatory infiltrate, proliferated and dilated vessels, and loss of the epidermal honeycomb structure.[74] Also characteristic are islands of tumour cells with peripheral palisading in the dermis, which delimit themselves from the dermis by a dark slit. This formation of an optical gap corresponds histologically to the accumulation of mucin. In one multi-centre study, a high sensitivity for CLM of 100% and a specificity of 88.5% in the diagnosis of basal carcinoma was able to be shown[1,24,27,34,40,43,65,70,74,75,86,91,96,97,99,117-119]

4.3.4 Other tumours

Among other things, in one pilot study, several criteria for the diagnosis of mycosis fungoides using CLM was able to be described. This includes hyporefractile papillae, atypical lymphocytes in the epidermis as well as at the junctional zone and in the dermis, Pautrier's microabscesses, blood vessel dilatation, and fibrosis. However, systematic studies investigating the applicability of these criteria are still lacking, especially with regard to the clinical differential diagnosis of eczema.[2,57]

Other studies include the description of other tumours and benign proliferations as single case descriptions or as smaller pilot studies, such as the CLM depiction of trichoepithelioma,[9] eccrine poroma,[105] disseminated superficial actinic porokeratosis,[110] sebaceous hyperplasia,[38] hydrocystoma,[117] and seborrheic keratosis.[6]

Other studies investigated vascular lesions and malformations with the help of CLM,[4,20,41,42,73] with characteristic CLM attributes being able to be defined in individual cases. What all these studies have in common is that systematic processing of the data in question as part of larger-scale studies and blinded analyses is still missing.

4.4 Inflammatory dermatosis

Under the bracket of inflammatory dermatosis, acute contact dermatitis and psoriasis are the skin diseases best studied using CLM.[12,17-19,36,37,46,57,95,103] Here, the characteristic features of spongiosis and vesicle formation have been described. The former appears as intercellular spaces in the epidermis with a lighter emphasis, while the latter appears as sharply delimited, partly septated dark cavities that appear with small or large lumina. Furthermore, reactive or concomitant inflammatory processes in the context of wound healing and tumours, or in the context of infections, [33,39,40,57,62,71,112] and also inflammation in autoimmune diseases, have been investigated.[10,13-15]

The inflammatory infiltrate itself appears as cells of varying refractility that are roundish to oval in shape and 8-10 µm, which can lie in the junctional, peripapillary or perivascular regions, or diffuse in the superficial dermis, depending on the disease process in the epidermis. Concomitant changes, e.g., of connective tissue in autoimmune processes, has also been able to be demonstrated with the help of CLM.[10,16] Again, assessment is limited by the optical depth of penetration, and precise differentiation of different immune cells is not currently possible. Finally, there are several reports on the study and therapeutic follow-up of cutaneous pigmentation disorders such as vitiligo or melasma[11,31,52-54.58, 64]

4.5 Other indications

Confocal laser microscopy is also suitable for diagnosis of pathogens. Fungal infections of the skin or nail body can be diagnosed directly on the patient without the need for tissue preparation. Hyphae and spores appear as brightly

reflective structures with typical morphology.[90]

Even mites such as Sarcoptes scabiei or Demodex folliculorum are clearly identifiable, making the method suitable for rapid diagnosis of scabies or for detecting and quantifying a Demodex infestation in rosacea.[61,62,92,94]

Bacterial and viral infections can only be detected indirectly through the morphology of the inflammatory reaction. The resolution is insufficient for depicting the pathogens. Herpes virus infections appear as typical acantholytic intra-epidermal vesicles. Bacterial infections are associated with accumulations of neutrophilic granulocytes.[33,35]

In the field of cosmetological research, confocal laser microscopy is used to objectify and quantify initial findings and therapeutic effects. Skin ageing is associated, among other things, with a flattening of the dermo-epidermal interface. This can be quantified in the confocal laser microscope on the basis of the density of the papillary tips in the horizontal section. Effects of UV irradiation and antioxidants, for example, can thus be studied.[56,66,68,72,89,95,106,112,120] In addition, there are some reports on photo-ageing, acne and acne scars and the follow-up care for these under dermatological therapy.[21,26,67]

4.6 Fluorescence diagnostics in vivo

With the VivaScope® Multilaser, fluorescence diagnostics is also possible in addition to reflectance imaging. Only a few fluorescent dyes are permitted for this, which are used in particular in ophthalmological diagnostics for intravenous application. Sodium fluorescein exhibits strong green fluorescence upon excitation at the 488 nm wavelength. Indocyanine green is excited at 785 nm and fluoresces in the near infrared region. Methylene blue and patent blue are used for in-vivo labelling of fistula tracts and sentinel diagnostics. These are red fluorescent dyes that become excited at 658 nm. The dyes can be applied topically to the skin surface and are sometimes still able to be detected in the skin folds and adnexa for many hours and days. They can be mixed with external agents and provide information about penetration paths and protective functions of topically applied

substances.[59,93102,104,107] Sodium fluorescein can also be injected intracutaneously, for which indocyanine green and the blue dyes are of limited use due to toxic effects and the risk of tattooing.[116] Systematic studies on in-vivo fluorescence diagnostics are still pending.

5. Ex-vivo confocal laser microscopy

Ex-vivo CLM is performed on pieces of freshly excised tissue. The resolution and layer thickness of ex-vivo confocal laser microscopy corresponds to that used for the in-vivo technique. Here, the maximum depth of penetration into the tissue is approximately 50 µm, so the outer surfaces of the tissue sections on the slide can be examined. In contrast to in-vivo examination, however, there is no limitation regarding the depth, since the tissue is placed on the cut surface, meaning that even dermal and subcutaneous structures can be displayed with the usual high resolution. The current main application of ex-vivo confocal laser microscopy is the examination of resection margins in tumour surgery by means of micrographically controlled surgery.[22,28,51,6375,97,121]

5.1 Examination technique

The examination can take place both in reflectance mode at 830 nm and in fluorescence mode at 488 and 658 nm (see below). With the VivaScope® 2500, the prepared tissue sections are placed on a slide with 0.9% NaCl solution and are fixed by means of a clamping device. After setting the best focus depth for clear imaging, the examination can then take place; analogous to the in-vivo method, scanning fields of size 630 µm x 630 µm are used here, which can be combined into fields of a freely selectable size (maximum 20 mm x 20 mm) via the software used in the device. Larger tissue sections can currently only be examined by manually moving the tissue on the slide.

The stored fields can then be assessed on the monitor either in the overview or using a zoom tool. Individual display of individual scan fields with high resolution is also possible.

5.1.1 Reflectance mode

As with in-vivo examination, the structures in the skin serve as an "endogenous" provider of contrast. In ex-vivo mode, these, in particular, include melanin and cellular structures – in particular the cell nuclei, but also collagen and elastic fibres. For contrast enhancement through protein precipitation (better reflectance), the unstained tissue is placed in 10% acetic acid or citric acid for about 1 minute and then washed down with NaCl solution. For reflectance mode, no further preparation of the tissue takes place, and this is then examined without staining, as described.

5.1.2 Fluorescence mode

In fluorescence mode, the wavelengths 488 nm and 658 nm are available for the excitation of fluorescent dyes introduced into the tissue; a filter system automatically blocks out the excitation wavelength in the device, so that only the fluorescent light is used for imaging. The aim is to selectively represent the individual structures (for example, cell nuclei in tumour cell diagnostics) by having the dyes bind as specifically as possible to them.
In fluorescence technology, after prior contrast enhancement by means of protein precipitation, as described above, the tissue is then deposited in the fluorescent dyes, so that the fluorophores can diffuse into the uppermost tissue layers and subsequently be used for fluorescence imaging.

5.1.2.1 Fluorescent dyes for *ex-vivo* application

Since when using the ex-vivo fluorescence technique, tissue toxicity and the risk of permanent tattooing at the hands of the fluorophores can be disregarded, additional dyes are available as compared to in-vivo examination. For excitation at 488 nm, sodium fluorescein and acridine orange are suitable, for the 658 nm laser, methylene blue, toluidine blue, patent blue and Nile blue.
The concentrations of the fluorophores are crucial for optimal results, since at too low concentrations, the fluorescence signal becomes too low, and at too high

concentrations, so-called quenching can occur, which results in images that are too dark. In addition, photobleaching can occur, an effect that is seen especially with methylene blue – to avoid this, one should strive to keep the scanning and measuring times as fast as possible. Some dyes – such as Nile blue – exhibit a pronounced dependence on pH levels, which can lead to a shift in the excitation and emission wavelengths. Recommendations for the concentrations of the various dyes have been determined for ex-vivo application based on various dilution measurement series.[116] An overview is given in Table 2.

Tab. 2

Fluorescence dye	**Recommended concentration**	**Challenges**	**Advantages**
Fluorescein	0.2% - 0.4%	Quenching, spectral absorption strongly pH-dependent	No tissue toxicity
Methylene blue	2 mg/ml	Photobleaching, permanent tattooing possible	No tissue toxicity
Patent blue	< 6 mg/ml (0.4 mg/ml)	Blue staining possible for up to 48h	No tissue toxicity
Acridine orange	1.2 mg/ml	Only for ex-vivo use due to potential carcinogenicity/ tissue toxicity	Good contrasting of epithelium against the stroma
Nile blue	0.2 mg/ml in eth. 70%	pH-dependent and solvent-dependent	Good contrasting o epithelium against the stroma
Indocyanine green	0.5 % (i.c.)	Tissue toxicity	Long lasting fluorescence for up to 48 hrs.

Tab. 2 Fluorophores for Fluorescence Diagnostics with Recommended Concentrations and Specific Features (according to Welzel et al, 2016).

5.2 Indications

The main indication for ex-vivo confocal microscopy is currently the examination of marginal sections in the context of micrographically controlled surgery for tumour excisions and frozen-section diagnostics. A big advantage of this method is an extremely short

examination time (about 5 minutes per image) compared to previous resection margin control procedures.

Preliminary studies to evaluate resection margin control using the confocal microscope were initially only performed in reflectance mode. In 2009, based on almost 250 resection margin images, Schüle et al described the examination technique as having a sensitivity of between 0% and 94% and a specificity of between 30% and 100%, depending on the resection technique (midsections, marginal sections or "muffins"). In a second series of 312 images, Ziefler et al. determined a sensitivity of between 73% and 94% and a specificity of between 36% and 78% in 2010.[121] Käb et al. arrived at a specificity of 90% and a sensitivity of 82% after examining 134 marginal and deep specimens.[51]

These data show that at the present time, the method certainly cannot completely replace HE histology in micrographically controlled surgery. Nevertheless, resection margins that are tumour-positive in confocal examination could establish the indication for subsequent excision in good time, thus significantly accelerating therapy.

A further approach is fluorescence diagnostics. Using the above-mentioned dyes, predominantly acridine orange, which causes tumour cells to stand out in high contrast, Karen et al. arrived at a specificity of 89.2% and a sensitivity of 96.6% in 2009, based on 149 confocal images, making this methodology interesting for clinical use.[55] In further studies, the high correlation between histopathology and FCM was able to be demonstrated,[45] and the method's suitability was evaluated and confirmed in 64 and 69 basal cell carcinomas, respectively.[22,63] Another approach could be coupling fluorescent dyes with specific antibodies (for basal cell carcinoma, e.g., BerEp4).

Digital staining

By combining the reflectance and fluorescence mode images and transforming the different grey levels by means of false colour coding using special software, it is possible to perform digital staining on the ex-vivo images which imitates and is similar to haematoxylin-eosin staining (H&E staining).[23] An evaluation of whether this procedure

increases sensitivity and specificity, especially when looking for small tumour nests or islands, remains the subject of further studies.

6. Limitations

Generally, CLM requires detailed histological knowledge of dermatological histology and pathology in order to correctly interpret the images, so this technique should definitely be learned in the form of training courses. There is also the possibility of networking with experts in CLM, who are available to give a second opinion. The horizontal images predominantly make diagnosis harder for histopathologists who are used to deep section images. However, its fusion with dermatoscopy in turn facilitates the attribution of pathological changes in CLM.

Although in-vivo CLM admittedly portrays individual cells in an almost microscopic resolution, these are only to be attributed on the basis of their reflection behaviour and their shape. Melanocytes and Langerhans cells are both highly reflective and have long dendrites, so these two cell types are usually distinguished only by their position within the epidermis. As a result, misinterpretations can occur, however, since in the case of melanomas, pagetoid-ascending melanocytes are also found in higher layers of the epidermis. In the case of tumours and inflammation, the dermo-epidermal junction zone is often coalesced and poorly defined, making it difficult to attribute the changes to a particular anatomical layer. Here, specifying the depth of the planes in relation to the previously defined zero point on the skin's surface is helpful. Measuring the vertical thickness of tumours is not possible in the horizontal images but can only be attempted on the stacks.

The biggest limitation of CLM is its low depth of penetration into the stratum papillare of the dermis. Thus, all deeper dermal alterations such as nodular melanoma, nodular basal cell carcinoma or panniculitis evade confocal diagnostics. It is only suitable for the diagnosis of diseases and tumours that exhibit their characteristic changes in the epidermis and upper dermis.

Likewise, the relatively long measurement duration and the small field of view can lead to limitations. Patients must be able to stay still for a few minutes. Strongly

curved, sunken, keratotic or weeping skin alterations are difficult to measure because the surface is not flush in one plane, or surface changes lead to artefacts and signal shadowing. Recording multiple lesions or even examining an entire skin field is possible in isolated cases at best with the VivaScope® 3000 handpiece.

7. Comparison with other methods

High-frequency 20 MHz sonography enables the display of deep-section images at a resolution of 80 to 200 µm and a penetration depth of up to 6 mm. It therefore also reveals dermal and subcutaneous changes, but does not allow cellular resolution and thus no differential diagnosis of tumours. Its domain is measuring the thickness of tumours and progress monitoring for collagenosis.

Optical coherence tomography (OCT) is an optical method that is similar to CLM. The resolution of OCT is slightly below that of CLM at 7 µm, which is usually insufficient for cellular diagnostics. Deep-section images with a penetration depth of 1.5 mm and parallel horizontal images are depicted. OCT is used for the diagnosis of basal cell carcinomas and actinic keratoses and is here superior to CLM because of the larger fields of view and significantly shorter measurement times, though it is not yet suitable for the differential diagnosis of pigmented lesions.

Multiphoton tomography is, similar to CLM, a technique that produces horizontal near-surface images, but with even better resolution in the subcellular region. However, thus far, the measurement process is even more complicated and the device significantly more expensive, and so the technology is not yet used as routine.

8. Literature

1. Agero AL, Busam KJ, Benvenuto-Andrade C, Scope A, Gill M, Marghoob AA, González S, Halpern AC. “Reflectance Confocal Microscopy of Pigmented Basal Cell Carcinoma”. J Am Acad Dermatol 2006; 54(4):638-43.
2. Agero AL, Gill M, Ardigo M, Myskowski P, Halpern AC, González S. In vivo reflectance confocal microscopy of mycosis fungoides: A preliminary study. J Am Acad Dermatol 2007;57(3):435-41
3. Aghassi D, Anderson RR, Gonzalez S. Confocal laser microscopic imaging of actinic keratoses in vivo: a preliminary report. J Am Acad Dermatol 2000;43(1 Pt 1):42-8
4. Aghassi D, Anderson RR, González S. Time-sequence histologic imaging of laser-treated cherry angiomas with in vivo confocal microscopy. J Am Acad Dermatol 2000 Jul;43(1 Pt 1):37-41
5. Ahlgrimm-Siess V, Massone C, Koller S, Fink-Puches R, Richtig E, Wolf I, Gerger A, Hofmann-Wellenhof R. In vivo confocal scanning laser microscopy of common naevi with globular, homogeneous and reticular pattern in dermoscopy. Br J Dermatol 2008;158(5):1000-7
6. Ahlgrimm-Siess V, Cao T, Oliviero M, Hofmann-Wellenhof R, Rabinovitz HS, Scope A. The vasculature of nonmelanocytic skin tumors in reflectance confocal microscopy, II: Vascular features of seborrheic keratosis. Arch Dermatol 2010 Jun;146(6):694-5
7. Alarcon I, Carrera C, Palou J, Alos L, Malvehy J, Puig S. Impact of in vivo reflectance confocal microscopy on the number needed to treat melanoma in doubtful lesions. Br J Dermatol 2014; 170(4):802-8
8. Antoniou C, Lademann J, Richter H, Astner S, Patzelt A, Zastrow L, Sterry W, Koch S. Analysis of the melanin distribution in different ethnic groups by in vivo laser scanning microscopy. Laser Phys Lett 2009; 6(5):393-398
9. Ardigo M, Zieff J, Scope A, Gill M, Spencer P, Deng L, Marghoob AA. Dermoscopic and reflectance confocal microscope findings of trichoepithelioma. Dermatology 2007;215(4):354-8
10. Ardigo M, Maliszewski I, Cota C, Scope A, Sacerdoti G, González S, Berardesca E. “Preliminary Evaluation of In Vivo Reflectance ConfocalMicroscopy Features of Discoid Erythematosus”. Br J Dermatol 2007; 156(6):1196-203
11. Ardigo M, Malizewsky I, Dell'anna ML, Berardesca E, Picardo M. Preliminary evaluation of vitiligo using in vivo reflectance confocal microscopy. J Eur Acad Dermatol Venereol 2007 Nov;21(10):1344-50
12. Ardigo M, Cota C, Berardesca E, González S. “Concordance between in vivo reflectance confocal microscopy and histology in the evaluation of plaque psoriasis.” J Eur Acad Dermatol Venereol 2009; 23(6):660-7
13. Ardigò M, Agozzino M, Amorosi B, Moscarella E, Cota C, de Abreu L, Berardesca E.Real-time, non-invasive microscopic confirmation of clinical diagnosis of bullous pemphigoid using in vivo reflectance confocal microscopy. Skin Res Technol 2014 May; 20(2): 194-199
14. Ardigo M, Donadio C, Franceschini C, Catricalà C, Agozzino M. Interest of reflectance confocal microscopy for inflammatory oral mucosal diseases. J Eur Acad Dermatol Venereol 2015; 29(9):1850-3
15. Ardigo M, Longo C, Gonzalez S; International Confocal Working Group Inflammatory Skin Diseases Project. Multicentre study on inflammatory skin diseases from The International Confocal Working Group: specific confocal microscopy features and an algorithmic method of diagnosis. Br J Dermatol

2016; 175(2):364-74

16. Ardigò M, Agozzino M, Franceschini C, Lacarrubba F.Reflectance Confocal Microscopy Algorithms for Inflammatory and Hair Diseases. Dermatol Clin 2016; 34(4): 487-496
17. Astner S, Gonzalez E, Cheung AC, Rius-Diaz F, González S. Pilot Study on the Sensitivity and Specificity of in-vivo Reflectance Confocal Microscopy in the diagnosis of Allergic Contact Dermatitis. J Am Acad Dermatol 2005;53(6):986-92
18. Astner S, González S, Gonzalez E. Non-Invasive evaluation of Allergic and Irritant Contact Dermatitis by in-vivo reflectance confocal microscopy. Dermatitis 2006;17(4):182-91
19. Astner S, Burnett N, Cheung AC, Rius- Díaz F, Doukas AG, González S and González E. The impact of skin color on the susceptibility to irritant contact dermatitis: a non-invasive evaluation. J Am Acad Dermatol 2006; 54:458-65
20. Astner S, González S, Cuevas J, Röwert-Huber J, Sterry W, Stockfleth E, Ulrich M. Preliminary evaluation of benign vascular lesions using in vivo reflectance confocal microscopy. Dermatol Surg 2010 Jul;36(7):1099-110
21. Bencini PL, Tourlaki A, Galimberti M, Longo C, Pellacani G, De Giorgi V, Guerriero G.Nonablative fractional photothermolysis for acne scars: clinical and in vivo microscopic documentation of treatment efficacy. Dermatol Ther 2012; 25(5): 463-467
22. Bennàssar A, Carrera C, Puig S, Vilalta A, Malvehy J. Fast evaluation of 69 basal cell carcinoma with ex vivo fluorescence confocal microscopy: criteria description, histopathological correlation and interobserver agreement. JAMA Dermatol 2013;149(7):839-847
23. Bini J, Spain J, Nehal k, Hazelwood, V, DiMarzio C, Rajadhyaksha M. Confocal mosaicing microscopy of human skin ex vivo: spectral analysis for digital staining to simulate histology-like appearance. J Biomed Opt. 2011;16:076008.
24. Braga JC, Scope A, Klaz I, Mecca P, González S, Rabinovitz H, Marghoob AA. The significance of reflectance confocal microscopy in the assessment of solitary pink skin lesions. J Am Acad Dermatol 2009; 61(2):230-41
25. Calzavara-Pinton P, Longo C, Venturini M, Sala R, Pellacani G. Reflectance confocal microscopy for in vivo skin imaging. Photochem Photobiol 2008 Nov-Dec;84(6):1421-30
26. Cameli N, Mariano M, Serio M, Ardigò M.Preliminary comparison of fractional laser with fractional laser plus radiofrequency for the treatment of acne scars and photoaging. Dermatol Surg 2014; 40(5): 553-561
27. Castro RP, Stephens A, Fraga-Braghiroli NA, Oliviero MC, Rezze GG, Rabinovitz H, Scope A. Accuracy of in vivo confocal microscopy for diagnosis of basal cell carcinoma: a comparative study between handheld and wideprobe confocal imaging. J Eur Acad Dermatol Venereol 2015; 29(6):1164-9
28. Chung VQ, Dwyer PJ, Nehal KS, Rajadhyaksha M, Menaker GM, Charles C, Jiang SB. Use of ex vivo confocal scanning laser microscopy during Mohs surgery for nonmelanoma skin cancers. Dermatol Surg 2004;30(12 Pt 1):1470-8
29. Cinotti E, Jaffelin C, Charriere V, Bajard P, Labeille B, Witkowski A, Cambazard F, Perrot JL. Sensitivity of handheld reflectance confocalmicroscopy for the diagnosis of basal cell carcinoma: A series of 344 histologically proven lesions. J Am Acad Dermatol 2015; 73(2):319-20
30. Cinotti E, Debarbieux S, Perrot JL, Labeille B, Long-Mira E, Habougit C, Douchet C, Depaepe L, Hammami-Ghorbel H, Lacour JP, Thomas L,

Cambazard F, Bahadoran P; Groupe Imagerie Cutanée Non Invasive de la Société Française de Dermatologie. Reflectance confocal microscopy features of acral lentiginous melanoma: a comparative study with acral nevi. J Eur Acad Dermatol Venereol 2016; 30(7):1125-8

31. Funasaka Y, Mayumi N, Asayama S, Takayama R, Kosaka M, Kato T, Kawana S. In vivo reflectance confocal microscopy for skin imaging in melasma. J Nippon Med Sch 2013; 80(3): 172-173
32. Gerger, A et al. Diagnostic applicability of in vivo confocal laser scanning microscopy in melanocytic skin tumors. J Invest Dermatol 2005;124(3):493-8
33. Goldgeier M, Alessi C, Muhlbauer JE: Immediate noninvasive diagnosis of herpesvirus by confocal scanning laser microscopy. J Am Acad Dermatol 2002, 46(5):783-785
34. Goldgeier M, Alessi-Fox C, Zavislan JM, Harris D, González S. “Noninvasive Imaging, Treatment and microscopic Confirmation of Clearance of Basal Cell Carcinoma”. Dermatol Surg 2003; 29(3):205-10
35. González S, Rajadhyaksha M, Gonzalez-Serva A, White WM, Anderson RR: Confocal reflectance imaging of folliculitis in vivo: correlation with routine histology. J Cutan Pathol 1999, 26(4):201-205
36. González S. "Characterization of Psoriasis In Vivo by Confocal Reflectance Microscopy". J Med 1999; 30(5-6):337-356
37. González S, González E, White WM, Rajadhyaksha M, Anderson, RR. “Allergic Contact Dermatitis: Correlation of In Vivo Confocal Imaging to Routine Histology”. J Am Acad Dermatol 1999; 40(5 Pt 1):708-13
38. González S, White WM, Rajadhyaksha M, Anderson RR, González E. Confocal imaging of sebaceous gland hyperplasia in vivo to assess efficacy and mechanism of pulsed dye laser treatment. Lasers Surg Med 1999;25(1):8-12
39. González S, Sackstein R, Anderson RR, Rajadhyaksha M. Real-time evidence of in vivo leukocyte trafficking in human skin by reflectance confocal microscopy. J Invest Dermatol 2001;117(2):384-386
40. González S, Tannous Z. “Real-time, In Vivo Confocal Reflectance Microscopy of Basal Cell Carcinoma”. J Am Acad Dermatol 2002; 47(6):869-74
41. Grazzini M, Stanganelli I, Rossari S, Gori A, Oranges T, Longo AS, Lotti T, Bencini PL, De Giorgi V. Dermoscopy, confocal laser microscopy, and hitech evaluation of vascular skin lesions: diagnostic and therapeutic perspectives. Dermatol Ther 2012; 25(4): 297-303
42. Grazziotin TC, Cota C, Buffon RB, Araújo Pinto L, Latini A, Ardigò M. Preliminary evaluation of in vivo reflectance confocal microscopy features of Kaposi's sarcoma. Dermatology 2010; 220(4): 346-354
43. Guitera P, Menzies SW, Longo C, Cesinaro AM, Scolyer RA, Pellacani G. In vivo confocal microscopy for diagnosis of melanoma and basal cell carcinoma using a two-step method: analysis of 710 consecutive clinically equivocal cases. J Invest Dermatol 2012; 132(10):2386-94
44. Guitera P, Menzies SW, Argenziano G, Longo C, Losi A, Drummond M, Scolyer RA, Pellacani G. Dermoscopy and in vivo confocal microscopy are complimentary techniques for the diagnosis of difficult amelanotic and light colored skin lesions. Br J Dermatol. 2016 Dec;175(6):1311-1319
45. Hartmann D, Ruini C, Mathemeier L, Dietrich A, Ruzicka T, von Braunmühl T. Identification of ex vivo confocal scanning microscopy features and their histological correlates in human skin. J Biophotonics 2016;9(4):376-87
46. Hicks SP, Swindells KJ, Middelkamp-Hup MA, Sifakis MA, Gonzalez E, Gonzalez S: Confocal histopathology of irritant contact dermatitis in vivo and

the impact of skin color (black vs white). J Am Acad Dermatol 2003, 48(5):727-734

47. Horn M, Gerger A, Koller S, Weger W, Langsenlehner U, Krippl P, Kerl H, Samonigg H, Smolle J. The use of confocal laser-scanning microscopy in microsurgery for invasive squamous cell carcinoma. Br J Dermatol 2007;156(1):81-4
48. Horn M, Gerger A, Ahlgrimm-Siess V, Weger W, Koller S, Kerl H, Samonigg H, Smolle J, Hofmann-Wellenhof R. Discrimination of actinic keratoses from normal skin with reflectance mode confocal microscopy. Dermatol Surg 2008;34(5):620-5
49. http://www.vivascope.de
50. Huzaira M, Rius F, Rajadhyaksha M, Anderson RR, González S. Topographic variations in normal skin, as viewed by in vivo reflectance confocal microscopy. J Invest Dermatol 2001;116(6):846-852
51. Käb S, Landthaler M, Hohenleutner U. Confocal laser scanning microscopy – evaluation of native tissue sections in micrographic surgery. Lasers Med Sci 2009; 24: 819-823
52. Kang HY, Bahadoran P, Ortonne JP. Reflectance confocal microscopy for pigmentary disorders. Exp Dermatol 2010 Mar;19(3):233-9
53. Kang HY, le Duff F, Passeron T, Lacour JP, Ortonne JP, Bahadoran P. A noninvasive technique, reflectance confocal microscopy, for the characterization of melanocyte loss in untreated and treated vitiligo lesions. J Am Acad Dermatol. 2010 Nov;63(5):e97-100
54. Kang HY, Bahadoran P. Application of in vivo reflectance confocal microscopy in melasma classification. J Am Acad Dermatol 2012 Jul; 67(1): 157-158
55. Karen JK, Gareau DS, Dusza SW, Tudisco M, Rajadhyaksha M, Nehal KS. Detection of basal cell carcinomas in Mohs excisions with fluorescence confocal mosaicing microscopy. Br J Dermatol 2009;160(6):1242-50
56. Kawasaki K, Yamanishi K, Yamada H.Age-related morphometric changes of inner structures of the skin assessed by in vivo reflectance confocal microscopy. Int J Dermatol. 2015; 54(3): 295-301
57. Koller S, Gerger A, Ahlgrimm –Siess V, Weger W, Smolle J, Hoffmann-Wellenhof R. In vivo reflectance confocal microscopy of erythrosquamous skin diseases. Exp Dermatol 2009;18(6)536-40
58. Lai LG, Xu AE. In vivo reflectance confocal microscopy imaging of vitiligo, nevus depigmentosus and nevus anemicus. Skin Res Technol 2011 Mar 24
59. Lange-Asschenfeldt B, Alborova A, Krüger-Corcoran D, Patzelt A, Richter H, Sterry W, Kramer A, Stockfleth E, Lademann J. Effects of a topically applied wound ointment on epidermal wound healing studied by in vivo fluorescence laser scanning microscopy analysis. J Biomed Opt 2009 Sep-Oct;14(5):054001
60. Langley RGB, Rajadhyashka M. Confocal scanning laser microscopy of benign and malignant melanocytic skin lesions in vivo. J Am Acad Dermatol. 2001;45(3): 365-76
61. Levi A, Mumcuoglu KY, Ingber A, Enk CD. Assessment of Sarcoptes scabiei viability in vivo by reflectance confocal microscopy. Lasers Med Sci 2011 May;26(3):291-2
62. 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 Oct;141(10):1336
63. Longo C, Rajadhyaksha M, Ragazzi M, Nehal K, Gardini S, Moscarella E,

Lallas A, Zalaudek I, Piana S, Argenziano G and Pellacani G. Evaluating ex vivo fluorescence confocal microscopy images of basal cell carcinomas in Mohs excised tissue. Br J Dermatol 2014; 171:561-570

64. Longo C, Pellacani G, Tourlaki A, Galimberti M, Bencini PL. Melasma and low-energy Q-switched laser: treatment assessment by means of in vivo confocal microscopy. Lasers Med Sci 2014; 29(3): 1159-1163
65. Longo C, Lallas A, Kyrgidis A, Rabinovitz H, Moscarella E, Ciardo S, Zalaudek I, Oliviero M, Losi A, Gonzalez S, Guitera P, Piana S, Argenziano G, Pellacani G. Classifying distinct basal cell carcinoma subtype by means of dermatoscopy and reflectance confocal microscopy. J Am Acad Dermatol 2014; 71(4):716-724.e1
66. Longo C. Well-aging: Early Detection of Skin Aging Signs. Dermatol Clin 2016; 34(4): 513-518
67. Lora V, Capitanio B, Ardigò M. Noninvasive, in vivo assessment of comedone re-formation. Skin Res Technol 2015; 21(3): 384-386
68. Ma YF, Yuan C, Jiang WC, Wang XL, Humbert P. Reflectance confocal microscopy for the evaluation of sensitive skin. Skin Res Technol. 2017 May;23(2):227-234
69. Malvehy J, Alarcon I, Montoya J, Rodríguez-Azeredo R, Puig S. Treatment monitoring of 0.5% 5-fluorouracil and 10% salicylic acid in clinical and subclinical actinic keratoses with the combination of optical coherence tomography and reflectance confocal microscopy. J Eur Acad Dermatol Venereol. 2016 Feb;30(2):258-65
70. Marra DE, Torres A, Schanbacher CF, Gonzalez S. Detection of residual basal cell carcinoma by in vivo confocal microscopy. Dermatol Surg 2005;31(5):538-41
71. Meyer LE, Otberg N, Tietz HJ, Sterry W, Lademann J. In vivo imaging of Malassezia yeasts on human skin using confocal laser scanning microscopy. Laser Physics Letters 2005; 2(3): 148-152
72. Middelkamp-Hup MA, Park HY, Lee J, Gilchrest BA, Gonzalez S: Detection of UV-induced pigmentary and epidermal changes over time using in vivo reflectance confocal microscopy. J Invest Dermatol 2006; 126(2):402-407
73. Moscarella E, Zalaudek I, Agozzino M, Vega H, Cota C, Catricalà C, Argenziano G, Ardigo M. Reflectance confocal microscopy for the evaluation of solitary red nodules. Dermatology 2012; 224(4); 295-300
74. Nori S, Rius-Díaz F, Cuevas J, Goldgeier M, Jaen P, Torres A, González S. Sensitivity and specificity of reflectance-mode confocal microscopy for in vivo diagnosis of basal cell carcinoma: a multicenter study. J Am Acad Dermatol 2004;51(6):923-30
75. Patel YG, Nehal KS. Confocal reflectance mosaicing of basal cell carcinomas in Mohs surgical skin excisions. J Biomed Opt 2007; 12(3): 034027
76. Pellacani G, Cesinaro AM, Seidenari S. In vivo confocal reflectance microscopy for the characterization of melanocytic nests and correlation with dermoscopy and histology. Br J Dermatol 2005;152:384-6
77. Pellacani G, Cesinaro A M, Seidenari S. “In Vivo Assessment of Melanocytic Nests in Nevi and Melanomas by Reflectance Confocal Microscopy”. Mod Pathol 2005; 18(4):469-74
78. Pellacani G, Cesinaro AM, Longo C et al. Microscopic in vivo description of cellular architecture of dermoscopic pigment network in nevi and melanomas. Arch Dermatol 2005; 141:147-54
79. Pellacani G, Cesinaro A.M., Seidenari S. “Reflectance-Mode Confocal Microscopy of Pigmented Skin Lesions – Improvement in Melanoma

Diagnostic Specificity". J Am Acad Dermatol 2005; 53(6):979-85

80. Pellacani G, Guitera P, Longo C et al. The impact of in vivo reflectance confocal microscope for imaging human tissue microscopy for the diagnostic accuracy of melanoma and equivocal melanocytic lesions. J Invest Dermatol 2007;127(12):2759-65
81. Pellacani G, Longo C, Malvehy J, Puig S, Carrera C, Segura S, Bassoli S, Seidenari S. "In Vivo Confocal Microscopic and Histopathologic Correlations of Dermoscopic Features in 202 Melanocytic Lesions". Arch Dermatol 2008;144(12):1597-1608
82. Pellacani G, Pepe P, Casari A, Longo C. Reflectance confocal microscopy as a second-level examination in skin oncology improves diagnostic accuracy and saves unnecessary excisions: a longitudinal prospective study. Br J Dermatol 2014; 171(5):1044-51
83. Pellacani G, De Pace B, Reggiani C, Cesinaro AM, Argenziano G, Zalaudek I, Soyer HP, Longo C. Distinct melanoma types based on reflectance confocal microscopy. Exp Dermatol 2014; 23(6):414-8
84. Pellacani G, Ulrich M, Casari A, Prow TW, Cannillo F, Benati E, Losi A, Cesinaro AM, Longo C, Argenziano G, Soyer HP. Grading keratinocyte atypia in actinic keratosis: a correlation of reflectance confocal microscopy and histopathology. J Eur Acad Dermatol Venereol 2015; 29(11):2216-21
85. Pellacani G, Witkowski A, Cesinaro AM, Losi A, Colombo GL, Campagna A, Longo C, Piana S, De Carvalho N, Giusti F, Farnetani F. Cost-benefit of reflectance confocal microscopy in the diagnostic performance of melanoma. J Eur Acad Dermatol Venereol 2016; 30(3):413-9
86. Peppelman M, Wolberink EA, Blokx WA, van de Kerkhof PC, van Erp PE, Gerritsen MJ. In vivo diagnosis of basal cell carcinoma subtype by reflectance confocal microscopy. Dermatology 2013; 227(3):255-62
87. Peppelman M, Nguyen KP, Hoogedoorn L, van Erp PE, Gerritsen MJ. Reflectance confocal microscopy: non-invasive distinction between actinic keratosis and squamous cell carcinoma. J Eur Acad Dermatol Venereol 2015; 29(7):1302-9
88. Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. J Invest Dermatol 1995;104: 946-52
89. Raphael AP, Kelf TA, Wurm EM, Zvyagin AV, Soyer HP, Prow TW.Computational characterization of reflectance confocal microscopy features reveals potential for automated photoageing assessment. Exp Dermatol 2013; 22(7): 458-463
90. Rothmund G, Sattler E, Kästle R, Fischer C, Haas CJ, Starz H, Welzel J. Confocal laser scanning microscopy as a new valuable tool in the diagnosis of onychomycosis - comparison of six diagnostic methods. Mycoses 2012, 56: 47-55
91. Ruocco E, Argenziano G, Pellacani G, Seidenari S. Noninvasive Imaging of Skin Tumors. Dermatol Surg 2004; 30(2 Pt 2):301-10
92. Sattler EC, Maier T, Hoffman VS, Hegyi J, Ruzicka T, Berking C. Noninvasive in vivo detection and quantification of Demodex mites by confocal laser scanning microscopy. Br J Dermatol 2012;167(5):1042-7
93. Sattler EC, Kästle R, Arens-Corell M, Welzel J. How long does protection last? - In vivo fluorescence confocal laser scanning imaging for the evaluation of the kinetics of a topically applied lotion in an everyday setting. Skin Res Technol. 2012; 18(3):370-7
94. Sattler EC, Hoffmann VS, Ruzicka T, Braunmühl T v, 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(1):69-75

95. Sauermann K, Gambichler T, Jaspers S, Radenhausen M, Rapp S, Reich S, Altmeyer P, Clemann S, Teichmann S, Ennen J, Hoffmann K. Histometric data obtained by in vivo confocal laser scanning microscopy in patients with systemic sclerosis. BMC Dermatol 2002;6:2-8
96. Sauermann K, Gambichler T, Wilmert M, Rotterdam S, Strucker M, Altmeyer P, Hoffman K. “Investigation of basal Cell Carcinoma by Confocal Laser Scanning Microscopy In Vivo”. Skin Res Technol 2002; 8(3):141-7
97. Schüle D, Breuninger H, Schippert W, Dietz K, Möhrle M. Confocal laser scanning microscopy in micrographic surgery (three-dimensional histology) of basal cell carcinomas. Br J Dermatol 2009; 161: 691-720
98. Scope A, Benvenuto-Andrade C, Agero A, Malvehy J, Puig S, Rajadhyaksha M, Busam K, Marra D, Torres A, Propperova I, Langley R, Marghoob A, Pellacani G, Seidenari S, Halpern A, González S. "In Vivo Reflectance Confocal Microscopy Imaging of Melanocytic Skin Lesions: Consensus Terminology, Glossary and Illustrative Images". J Am Acad Dermatol 2007; 57:644-58
99. Segura S, Puig S, Carrera C, Palou J, Malvehy J. Dendritic cells in pigmented basal cell carcinoma: a relevant finding by reflectance-mode confocal microscopy. Arch Dermatol 2007;143(7):883-6
100. Segura S, Puig S, Carrera C, Palou J, Malvehy J. Development of a two-step method for the diagnosis of melanoma by reflectance confocal microscopy. J Am Acad Dermatol 2009; 61(2):216-29
101. Song E, Grant-Kels JM, Swede H, D'Antonio JL, Lachance A, Dadras SS, Kristjansson AK, Ferenczi K, Makkar HS, Rothe MJ. Paired comparison of the sensitivity and specificity of multispectral digital skin lesion analysis and reflectance confocal microscopy in the detection of melanoma in vivo: A cross-sectional study. J Am Acad Dermatol 2016 Sep 29. pii: S0190-9622(16)30500-X
102. Suihko C, Swindle LD, Thomas SG, Serup J. Fluorescence fibre-optic confocal microscopy of skin in vivo: microscope and fluorophores. Skin Res Technol 2005 Nov;11(4):254-67
103. Swindells K, Burnett N, Rius-Diaz F, González E, Mihm MC, González S. Reflectance confocal microscopy may differentiate acute allergic and irritant ontact dermatitis in vivo. J Am Acad Dermatol 2004;50:220-228
104. Swindle LD, Thomas SG, Freeman M, Delaney PM. View of normal human skin in vivo as observed using fluorescent fiber-optic confocal microscopic imaging. J Invest Dermatol 2003 Oct;121(4):706-12
105. Tachihara R, Choi C, Langley RG, Anderson RR, González S. In vivo confocal imaging of pigmented eccrine poroma. Dermatology 2002;204(3):185-9
106. Tan JM, Lambie D, Sinnya S, Sahebian A, Soyer HP, Prow TW, Ardigò M. Histopathology and reflectance confocal microscopy features of photodamaged skin and actinic keratosis. J Eur Acad Dermatol Venereol 2016; 30(11): 1901-1911
107. Teichmann A, Heuschkel S, Jacobi U, Presse G, Neubert RH, Sterry W, Lademann J. Comparison of stratum corneum penetration and localization of a lipophilic model drug applied in an o/w microemulsion and an amphiphilic cream. Eur J Pharm Biopharm. 2007 Nov;67(3):699-706
108. Ulrich M, Stockfleth E, Roewert J and Astner S. Noninvasive diagnostic tools for nonmelanoma skin cancer. Br J Dermatol 2007, 157 S2:56-8

109. Ulrich M, Maltusch A, Röwert J, González S, Sterry W, Stockfleth E, Astner S. Actinic keratoses: non- invasive diagnosis for field cancerisation. Br J Dermatol 2007; 156(3):13-17
110. Ulrich M, Forschner T, Röwert J, González S, Stockfleth E, Sterry W, Astner S. Differentiation between actinic keratoses and disseminated superficial actinic porokeratoses with reflectance confocal microscopy. Br J Dermatol 2007; 156(3):47-52
111. Ulrich M, Maltusch A, Rius-Diaz F, Röwert J, González S, Sterry W, Stockfleth E, Astner S. Clinical Applicability of in vivo Reflectance Confocal Microscopy for the Diagnosis of Actinic Keratoses. J Dermatol Surg, 2008;34(5):610-9
112. Ulrich M, Rüter C, Astner S, Sterry W, Lange-Asschenfeldt B, Stockfleth E, Röwert-Huber J. Comparison of UV-induced skin changes in sun-exposed vs. sun-protected skin- preliminary evaluation by reflectance confocal microscopy. Br J Dermatol 2009 Nov;161 Suppl 3:46-53
113. Ulrich M, Krueger-Corcoran D, Roewert-Huber J, Sterry W, Stockfleth E, Astner S. Reflectance Confocal Microscopy for Noninvasive Monitoring of Therapy and Detection of Subclinical Actinic Keratoses. Dermatology 2010;220(1):15-24
114. Ulrich M, González S, Lange-Asschenfeldt B, Roewert-Huber J, Sterry W, Stockfleth E, Astner S. Non-invasive diagnosis and monitoring of actinic cheilitis with reflectance confocal microscopy. J Eur Acad Dermatol Venereol 2011 Mar;25(3):276-84
115. Ulrich M, Reinhold U, Skov T, Søndergaard RE, Guitera P. Histological examination confirms clinical clearance of AKs following treatment with ingenol mebutate 0.05% gel. Br J Dermatol. 2017 Jan;176(1):71-80
116. Welzel J, Kästle R, Sattler EC. Fluorescence (multiwave) confocal microscopy. Dermatol Clin 2016 Oct, 34(4): 527-533
117. Willard K, Warschaw KE, Swanson DL. Use of reflectance confocal microscopy to differentiate hidrocystoma from Basal cell carcinoma. Dermatol Surg 2011 Mar;37(3):392-4
118. Witkowski AM, Łudzik J, DeCarvalho N, Ciardo S, Longo C, DiNardo A, Pellacani G. Non-invasive diagnosis of pink basal cell carcinoma: how much can we rely on dermoscopy and reflectance confocal microscopy? Skin Res Technol 2016; 22(2):230-7
119. Xiong YD, Ma S, Li X, Zhong X, Duan C, Chen Q. A meta-analysis of reflectance confocal microscopy for the diagnosis of malignant skin tumours. Eur Acad Dermatol Venereol 2016; 30(8):1295-302
120. Yamashita T, Akita H, Astner S, Lerner EA, González S. The evaluation of the melanin and blood flow changes in UVA irradiated skin by reflectancemode confocal microscopy. Exp Dermatol 2007; 16:905-911
121. Ziefle S, Schüle D, Breuninger H, Schippert W, Möhrle M. Confocal laser scanning microscopy vs 3-dimensional histologic imaging in basal cell carcinoma. Arch Dermatol 2010; 146(8): 843-847

Declaration of conflicts of interest

The following possible conflicts of interest of the authors regarding the guideline report were recorded:

		Julia Welzel	Susanne Lange-Asschenfeldt	Martina Ulrich	Wilhelm Stolz	Elke Sattler
1	Activities as a consultant or expert reviewer or paid participation in a scientific advisory board of a healthcare company (e.g., pharmaceutical industry, medical devices industry), a commercially oriented contract research organisation or an insurance provider	Expert assessment on the efficacy and tolerability of cosmetic products, consultancy activities for the company Unilever, the company Michelson, and the company DermaLumics	Consultancy activities for the company Mavig until 2007	Scientific consultancy activities for the company Oncobeta and the company Mavig	No	No
2	Fees for lecturing and training activities or paid authorships or co-authorships on behalf of a healthcare company, a commercially oriented contract research organisation or an insurance provider	Fees for lecturing activities from the companies Leo, Pohl- Boskamp Novartis, Almirall. Galderma	Fees for lecturing activities from the company Mavig until 2007	Fees for lecturing activities from the companies Almirall, Mavig	Fees for lectures from the companies Leo, Almirall, La Roche-Posay	Fees for lecturing activities from the company La Roche Posay

3	Financial contributions (third-party funds) for research projects or direct financing of the institutions' employees by a healthcare company, a commercially oriented contract research organisation or an insurance provider	No	No	No	No	No
4	Owner interest in pharmaceuticals/medical devices (e.g., patents, copyright, sales licence)	No	No	No	No	No
5	Ownership of shares, stocks, funds with participation in healthcare companies	Managing director and shareholder of DermaFocus GmbH (contract research organisation)	No	No	No	No
6	Personal relationships with an authorised representative of a healthcare company	No	No	No	No	No
7	Member of specialist societies/professional associations relevant to the development of the guidelines, elected representative within the context of the development of the guidelines	German Dermatological Society, Working Group for Physical Diagnostics in Dermatology	Working Group for Physical Diagnostics in Dermatology	Working Group for Dermatological Oncology, Working Group for Physical Diagnostics in Dermatology	German Dermatological Society, Working Group for Physical Diagnostics in Dermatology	German Dermatological Society, Working Group Physical Diagnostics in Dermatology

				Dermatology	Working Group for Dermatological Oncology	
8	Political, academic (e.g., affiliation with certain "schools"), scientific or personal interests that could give cause for possible conflicts of interest	Cooperation agreement with the company Mavig for scientific consultancy, provision of imaging material and conducting training regarding confocal laser microscopy (without personal benefits)	Conducting training for the company Mavig, scientific consultancy	Conducting training for the company Mavig, scientific consultancy	No	Provision of a Vivascope by the company Mavig for study purposes
9	Current employer, relevant previous employers of the last 3 years	Augsburg Hospital	Charité University Hospital in Berlin	Self-employed in own private practice and CMB Collegium Medicum Berlin GmbH; formerly: Charité University Hospital in Berlin	Munich Municipal Hospital	Hospital of the University of Munich (KUM)

These were discussed and evaluated together. The cooperation of the author Welzel with the company Mavig, which distributes the confocal laser microscopes in Germany, relates to scientific consultation, further development of the equipment, provision of imaging material and carrying out training and courses. The author Welzel

does not receive any personal financial benefits through this cooperation. The other authors have sometimes received fees for training and travel expenses and have sometimes received equipment free of charge. These benefits were based on appropriate services in return. There is currently only one supplier of devices for CLM. It is not possible to amass experience with CLM or pass this on without having relations to the company Mavig. The authors thus do not see any significant conflicts of interest.

Date of creation: 07/2011

Revised: 07/2017

Next review planned: 07/2022