Guideline: Confocal Laser Microscopy in Dermatology

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**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.

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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. 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. 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:

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

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 x 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 sized 1000 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.\textsuperscript{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 refractivity, corresponding to the varying melanin content of the Fitzpatrick phototypes. The melanin content correlates with the corresponding reflectivity and thus with the image brightness.\textsuperscript{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.\textsuperscript{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.

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.

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 shown1,24,27,34,40,43,65,70,74,75,86,91,96,97,99,117-119

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,\textsuperscript{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.\textsuperscript{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,\textsuperscript{33,39,40,57,62,71,112} and also inflammation in autoimmune diseases, have been investigated.\textsuperscript{10,13-15}

The inflammatory infiltrate itself appears as cells of varying refractility that are roundish to oval in shape and 8-10 $\mu$m, 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.\textsuperscript{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\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{21,26,67}

4.6 Fluorescence diagnostics in vivo

With the VivaScope\textregistered 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
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. 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.

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. An overview is given in Table 2.

Tab. 2

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

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


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### Declaration of conflicts of interest

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

<table>
<thead>
<tr>
<th></th>
<th>Julia Welzel</th>
<th>Susanne Lange-Asschenfeldt</th>
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<th>Elke Sattler</th>
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<td>1</td>
<td>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</td>
<td>Expert assessment on the efficacy and tolerability of cosmetic products, consultancy activities for the company Unilever, the company Michelson, and the company DermaLumics</td>
<td>Consultancy activities for the company Mavig until 2007</td>
<td>Scientific consultancy activities for the company Oncobeta and the company Mavig</td>
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<td>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</td>
<td>Fees for lecturing activities from the companies Leo, Pohl-Boskamp Novartis, Almirall. Galderma</td>
<td>Fees for lecturing activities from the company Mavig until 2007</td>
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</table>
### 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

<p>| German Dermatological Society, Working Group for Physical Diagnostics in Dermatology | German Dermatological Society, Working Group for Physical Diagnostics in Dermatology | German Dermatological Society, 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 | German Dermatological Society, Working Group Physical Diagnostics in Dermatology |</p>
<table>
<thead>
<tr>
<th>No</th>
<th>Category</th>
<th>Detail</th>
<th>Working Group for Dermatological Oncology</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Political, academic (e.g., affiliation with certain “schools”), scientific or personal interests that could give cause for possible conflicts of interest</td>
<td>Cooperation agreement with the company Mavig for scientific consultancy, provision of imaging material and conducting training regarding confocal laser microscopy (without personal benefits)</td>
<td>Conducting training for the company Mavig, scientific consultancy</td>
<td>Provision of a Vivascope by the company Mavig for study purposes</td>
</tr>
<tr>
<td>9</td>
<td>Current employer, relevant previous employers of the last 3 years</td>
<td>Augsburg Hospital</td>
<td>Charité University Hospital in Berlin</td>
<td>Munich Municipal Hospital</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Self-employed in own private practice and CMB Collegium Medicum Berlin GmbH; formerly: Charité University Hospital in Berlin</td>
<td>Charité University Hospital in Berlin</td>
<td>Munich Municipal Hospital</td>
</tr>
</tbody>
</table>

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.

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Next review planned: 07/2022

The "guidelines" of the Scientific Medical Societies are systematically developed aids for doctors to help them make decisions in specific situations. They are based on current scientific findings and proven best practices and ensure greater safety in medicine, but are intended to also take account of economic considerations. The "guidelines" are not legally binding for doctors and therefore neither give rise to any liability or release any party from liability.

The AWMF records and publishes the guidelines of the professional societies with the greatest possible care. Nevertheless, the AWMF cannot assume any responsibility for the accuracy of the content. With dosage information, in particular, the information provided by the manufacturer should always be heeded!