Confocal Laser Microscopy in Dermatology

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1. Introduction

Confocal laser microscopy is a noninvasive method for high-resolution diagnostics of tissue. While conventional microscopes work by using transmitted light technology, with thin tissue layers being illuminated from below, the confocal laser microscopes designed for dermatology work with incident light technology. The skin is illuminated from above with a focused laser. The reflected light is directed through a pinhole onto a detector so that only signals from a defined horizontal plane are used for high-resolution imaging. This technique limits the penetration depth into skin. Examinations are possible non-invasive in vivo and in real time. Confocal laser scanning microscopy allows in vivo microscopic imaging of skin layers close to the surface and opens up new possibilities for dermatology to diagnose and progression monitor, in particular in the case of dynamic changes. It also can be used ex vivo with freshly excised tissue, resembling a frozen section analysis, which is interesting especially for the field of microscopically controlled surgery of skin tumors.

For imaging, the reflected light signals from a horizontal plane are detected. The light is reflected at interfaces where the refractive index changes. Highly reflective structures of the skin are keratin, melanin, and collagen. When using monochromatic laser lights and a suitable filter, fluorescence can be used for imaging in addition to reflection. This requires that the skin be dyed from the outside with fluorescent dyes injected intradermally.

2. Devices

For confocal laser microscopy, devices with one or more lasers as the light source are utilized, which can be used for both in vivo and ex vivo examination of the skin. The specification of the device characteristics is governed by the Medical Device Regulation and the Medical Product Act. VivaScope ® devices are CE-certified according to ISO 13485:2003, ISO 9001:2008, as well as CE 0459. The laser energy on tissue level is less than 30 mW, so there is no risk for the tissue to be examined or the human eye (Laser Class I) (http://www.vivascope.de, Rajadhyaksha et al, JID, 1995; Rajadhyaksha et al, Applied Optics, 1999).

A confocal laser scanning microscope for in vivo diagnosis consists of different elements and generally includes an equipment cart and a PC with monitor, pivot arm, and laser unit. A macro camera to be connected is available as optional equipment. Commercially available devices have a variable penetration depth, which can be used to represent in high resolution superficial
dermal and epidermal structures, depending on the used wavelength. A magnetic ring is used to affix on skin local devices, so-called hand-held devices, which also enable examinations of difficult to access areas of skin such as the nose or the back of the knee. Currently, the VivaScope 1500®, VivaScope Multiwave 1500, and the VivaScope 3000® are in clinical practice or scientific testing (http://www.vivascope.de). The VivaScope 1500 Multiwave can be equipped with up to three lasers simultaneously. In addition to the light source at 830 nm, which is exclusively used for reflection diagnostics, lasers with 445 nm, 488 nm, 658 nm, and 785 nm are used for fluorescence and reflection diagnostics. The filters allow the depiction of this reflection alone, fluorescence alone, or reflection and fluorescence in one image. With the shorter wavelengths, the reflection images depict a higher resolution with lower penetration depth in near-surface sections. For fluorescence diagnostic, sodium fluorescein can be used in vivo at 445 nm and 488 nm, methylene and patent blue at 658 nm and indocyanine green at 785 nm as dyes. These can either be applied topically to the skin surface or injected intracutaneously.

The ex vivo confocal laser-scanning microscopy on the other side allows the examination of native tissue sections. For this purpose, the devices are configured somewhat differently: On a cart with PC and monitor, the confocal laser microscope is mounted underneath a cross-table with slide onto which the tissue sections to be examined are placed. Compared to the in vivo systems, the ex vivo system features an inverse layout. The tissue placed on the slide can be shifted with the cross-table in two horizontal axes so that diagnosing larger tissue samples is possible as well. The tissue samples are positioned on the cutting edges to enable examination of the depth sections of the entire tissue, contrary to the in vivo diagnostics.

As a device, only the VivaScope 2500 Multiwave system (ex vivo) is available presently, which features the standard wave length of 830 nm for the reflection as well as wave lengths of 445 nm and 658 nm (blue and red) to excite exogenous fluorescence dyes (http://www.vivascope.de).

3. Indications

Confocal laser scanning microscopy is suitable for dermatological, noninvasive diagnostic of near-surface skin changes. In the area of the skin tumors, it is especially of interest to assess melanocytic lesions with respect to their benign or malign character in order to enable the early detection of melanoma and to avoid unnecessary excisions. Non-invasive early detection of
epithelial tumors is important as well, as is progression and therapy monitoring, particularly if non-surgical topical therapies are used, which do not yield histologies.

Superficial inflammatory skin diseases can be examined with confocal laser scanning microscopy, too. In these cases, primary focus is usually not so much on diagnosis but on progression monitoring and quantification of therapy effects.

There are limitations to the methodology if tumors portions are very deep within the skin or if inflammation exists since these are difficult or impossible to depict due to the low penetration depth of CLSM. The horizontal imaging of the tissue layers requires a rethinking when interpreting the images, even for a histologist with experience in depth sections.

When used ex vivo, confocal laser scanning microscopy is suitable for the frozen section technique and microscopic excisional margin control. A great advantage over conventional histology is that time-consuming tissue preparation is not required so that results are available within a few minutes.

4. In vivo confocal laser microscopy

4.1 Examination technique

The optical principle of confocal laser scanning microscopy (CLSM) is comparable to that of ultrasound, where a point light source is used to depict skin structures in virtual, horizontal slices. The commercially available LSM (VivaScope 1500) is equipped with an 830 nm diode laser focused by the physician on any level within the tissue. A raster scanner is used to examine the tissue. The imaging of the LSM is based on the reflection and scattering of light from the examined tissue section. Differences in the refractive indices of the various cell structures and skin layers lead to differentiated reflection patterns, and these sections are depicted in different shades of gray (Rajadhyaksha et al, 1995; Rajadhyaksha et al, JID, 1999; Rajadhyaksha et al, Applied Optics, 1999; Huzaira et al, 2001; Calzavara-Pinton et al, 2008).

Melanin, hemoglobin, cellular microstructures, and collagen serve as "endogenous" contrast agents. Melanocytic lesions are therefore especially well imaged using LSM. Reflected light beyond the focal plane is filtered out by a very small aperture, which results in the high resolution of LSM. The lateral resolution is in the range of 0.5 to 1 μm in the cellular area, the axial resolution (layer thickness) of 3 to 5 μm corresponds to the layer thickness of normal histological examinations (Rajadhyaksha et al, 1995; Rajadhyaksha et al, JID, 1999; Rajadhyaksha et al, Applied Optics, 1999).
To reduce light refraction on the skin surface through the high refractive index of the stratum corneum, water or oil immersion lenses are used during the exam. Before positioning the ring on the skin, the lesion should be cleaned with isopropyl alcohol and a drop of oil (e.g. Crodamol oil) is to be added to the adhesive window. Alternatively, it is also possible to use water-based gels, especially if surfaces are slanted or areas are highly hyperkeratotic, since these prevent rapid run off and become thoroughly lodged between the detached corneocytes (Rajadhyaksha et al, 1995; Rajadhyaksha et al, JID, 1999; Rajadhyaksha et al, Applied Optics, 1999).

The use of a removable metal ring keeps the immersion medium and the lens stable during the exam process and thus minimizes the occurrence of motion artifacts. After applying the immersion medium, this ring is affixed to the skin of the patient with the help of adhesive stickers and the lens housing is attached magnetically. By moving the lens in the z (vertical) axis in relation to the skin surface, it is possible to examine different levels within the tissue. At the same time one can examine the tissue within a selected plane in the XY (horizontal) axis at a different dimension; individual images are 500 μm x 500 μm in size, individual summary folders between 1 mm x 1 mm to 8 mm x 8 mm. Image interpretation is based on virtual horizontal sections of the tissue, contrary to routine histology. The inclusion of smaller video sequences allows the documentation of dynamic processes such as blood flow or the migration of inflammatory cells (Rajadhyaksha et al, 1995; Rajadhyaksha et al, JID, 1999; Rajadhyaksha et al, Applied Optics, 1999; Gonzalez et al, JID, 2001; Astner et al, 2010; Astner et al, Dermatitis, 2006; Ahlgrimm-Siess et al, 2010).

The use of a digital macro-camera with dermatoscopic quality ("VivaCam") allows the macroscopic assessment of the skin areas to be examined. The VivaCam is placed over the standard ring of the VivaScope 1500 on the skin, thus allowing the correlation between the macroscopic and the image and the confocal images generated by the VivaScope. The individual images have a size of 5 Mpx and an image area of 10 mm. Since both devices are electronically linked, it is possible to navigate within the macroscopic image to specify areas for the subsequent microscopic viewing with the VivaScope (http://www.vivascope.de; Pellacani et al, BJD, 2005).

Integrated software enables systematic image documentation. A patient record can be created in the system for each patient, which is used to save patient data and information regarding the examined skin changes. Alternatively, it is also possible to store data in anonymous form. Due to the limited capacity of the local hard disk, it is necessary to have available additional storage in the form of an external hard drive or server system. This also ensures that backup copies of
the image files are created. The storage of individual frames or XY maps (Viva Block®) or Z-blocks (Viva Stack®) makes it possible to re-evaluate the images at a later date or obtain a second opinion (http://www.vivascope.de).

4.2 Description of findings

The description of findings with the help of confocal laser microscopy depends on the skin region to be examined. In general, the exam begins in the uppermost skin layers with the depiction of the stratum corneum, followed by the other layers of the epidermis (stratum granulosum, stratum spinosum, suprapapillary region, junction zone, and dermis). At the same time, physicians can examine the tissue within a selected plane in the horizontal (xy) axis in different dimensions. Here, individual survey maps with a size of between 1 mm x 1 mm to 8 mm x 8 mm are prepared, each consisting of 2 x 2 or up to 16 x 16 individual frames, which in turn are stored as an overview and individual images. Dynamic processes within the examined tissue section can be documented with smaller video sequences.

4.3 Depiction of healthy skin

The stratum corneum is displayed first when examining healthy areas of skin (Huzaira et al, 2001). The polygonal anucleate corneocytes form a cohesive, highly refractile cell structure with the markings, creases, and wrinkles typical for healthy skin, which appear as dark lines between the aggregated corneocytes. LSM depicts individual corneocytes with a size of 20 to 30 μm (Huzaira et al, 2001; Calzavara-Pinton et al, 2008).

The stratum granulosum is depicted underneath, consisting of 2-4 cell layers with a single cell size between 20 μm and 25 μm. The centrally located nuclei are shown as dark, oval-rounded structures surrounded by a narrow ring with a bright cytoplasmic 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 suggest the first pigmented basal cells of the papillae in some areas, discernible by a cobblestone-like pattern. The basal cell layer itself consists of more or less heavily refractile cells, according to the different melanin content of the light types according to Fitzpatrick. Here, the melanin content correlates with the corresponding reflectivity and thus the image brightness (Middelkamp-Hup et al, 2006; Yamashita et al, 2007; Antoniou et al, Laser Phys Lett, 2009). The cell size is between 10 and 12 μm. In the dermoepidermal junction zone, the basal cells form bright rings around the
centrally located, dark papillae. Within the papillae tips, the blood flow of superficial capillaries is usually depicted (Middelkamp-Hup et al, 2006; Yamashita T et al, 2007; Antoniou C et al, Laser Phys Lett, 2009). Below the junction zone, the reticular bundles of the dermal connective tissue appear, with topographical and age-related differences in arrangement, density, and reflectivity.

Skin adnexa such as hair follicles, sebaceous glands, and ducts of eccrine glands can also be mapped using LSM. Eccrine ducts appear as a light spiral-like structure in the epidermis; sebaceous glands appear as round, coil-like formations, with a central upright hair, which presents itself as a linear, highly refractile structure with visible layering.

Other topographical differences consist between hairy skin and hairless skin of palms and soles. The latter has also a much thicker stratum corneum, which is measurable with a micrometer, and demonstrates the regular distribution of pore-like openings of the eccrine glands, which LSM depicts as dark areas (Huzaira et al, 2001).

4.4 Tumors

Confocal laser scanning microscopy was used already during the early stages of development concerning the diagnosis of neoplastic skin changes, where the emphasis was on the examination of malignant melanoma and its distinction from benign melanocytic proliferations. Another focus was the examination of benign skin lesions, with definition of the LSM criteria of actinic keratoses, basal cell carcinoma, as well as related disease patterns such as Bowen's disease (Ahlgrimm-Siess et al, 2008; Ahlgrimm-Siess et al, 2010; Agero et al, 2006; Aghassi et al, 2000; Gerger et al, JID, 2005; Goldgeier et al, 2003; Gonzalez and Tannous, 2002; Horn et al, 2007; Horn et al, 2008; Karen JK et al, 2009; Langley and Rajadhyaksha, 2001; Marra DE et al 2005, Nori et al, JAAD, 2004; Ruocco et al, Dermatol Surg, 2004; Pellacani et al, BJD, 2005; Pellacani et al, Mod Pathol, 2005; Pellacani et al, Arch Dermatol, 2005; Pellacani et al, JAAD, 2005; Pellacani et al, JID, 2007; Pellacani et al, Arch Dermatol, 2008; Sauermann et al, Skin Res Technol, 2002; Ulrich et al, BJD, 2007; Ulrich et al, BJD, 2007; Ulrich et al, BJD, 2008).

4.4.1 Malignant melanoma

Malignant melanoma has been an early subject of systematic confocal laser microscopy studies because melanocytic lesions can be depicted with great definition due to the clear
endogenous contrast of melanin. In recent years, the clinical applicability of LSM for melanoma diagnosis has been proven with numerous studies. Defined morphological imaging characteristics of melanoma and benign melanocytic lesions were compiled in this context. The most important melanoma criteria are here the removal of the normal epidermis architecture, lack of delineation of the papillae (so-called non-edged papillae), irregular nests of atypical melanocytes, and the presence of large and highly refractile cells with prominent nucleus in higher epidermal layers (Pellacani et al, BJD, 2005; Pellacani et al, Mod Pathol, 2005; Pellacani et al, Arch Dermatol, 2005; Pellacani et al, JAAD 2005; Pellacani et al, JID, 2007; Pellacani et al, Arch Dermatol, 2008; Gerger et al, 2005; Ahlgrimm-Siess et al, 2008; Langley and Rajadhyaksha, 2001; Scope et al, JAAD, 2007).

4.4.2 Actinic keratoses

In LSM, actinic keratoses are characterized by a loss of the normal honeycomb structure with atypia and pleomorphism of epidermal keratinocytes, parakeratosis, detached corneocytes in the stratum corneum, and solar elastosis, as well as blood vessel dilatation. Horizontal overview maps frequently show superimposed compact hyperkeratosis, making the visualization of deeper structures in some cases significantly more difficult. This is also the reason for the restriction of LSM concerning the diagnosis of invasive squamous cell carcinoma since here severe hyperkeratosis often limits the evaluation of the lesions (Aghassi et al, 2000; Ulrich et al, 2007; Ulrich et al, 2007; Ulrich et al, JEADV, 2011; Ulrich et al, Dermatology, 2010; Horn M et al, 2007; Horn M et al, 2008; Ahlgrimm-Siess et al, Arch Dermatol, 2010; Segura et al, 2007; Ziefle et al, 2010).

4.4.3 Basal cell carcinoma

When using LSM, basal cell carcinoma exhibit characteristic changes, with the following five main criteria having been described: elongated, monomorphic nuclei; polarization of these cells along an axis; pronounced inflammatory infiltrate; increased as well as dilated blood vessels; and loss of epidermal honeycomb structure (Nori et al 2004). In addition, tumor cell islands with peripheral palisading, distinguishable from the dermis by a dark gap, are often identified in the dermis as well. This optical gap formation corresponds histologically to the accumulation of mucin (Gonzalez et al, 2002; Goldgeier et al, 2003; Willard et al, 2011; Schüle

4.4.4 Others

Among other findings, a pilot study was able to describe some criteria for the LSM diagnosis of mycosis fungoides. These include hyporefractile papillae, atypical lymphocytes in the epidermis and at the junction and in the dermis, Pautrier's microabsceses, blood vessel dilatation, and fibrosis. However, there is currently still a lack of systematic studies that examine the applicability of these criteria, particularly in regard to the clinical differential diagnosis of eczema disorders (Agero et al, 2007; Koller S et al, 2009). Further studies present the description of other tumors and benign proliferations as individual case descriptions or minor pilot studies, such as the LSM mapping of trichoepithelioma (Ardigo et al, 2007); eccrine poromerics (Tachihara et al, 2002) disseminated, superficial actinic porokeratosis (Ulrich et al, BJD, 2007); sebaceous gland hyperplasia (Gonzalez et al, Lasers Surg Med, 1999) of hidrocystomas (Willard et al, Dermatol Surg, 2011); and seborrheic keratoses (Ahlgrimm-Siess et al, Arch Dermatol, 2010). Other studies have examined vascular lesions and malformations with the help of LSM (Aghassi D et al, JAAD, 2000; Astner et al, Dermatitis, 2006) where characteristic features of LSM could be defined in individual cases. All these studies have in common that a systematic review of the relevant data in larger-scale studies and blind analyses still must be carried out.

4.5 Inflammatory dermatoses

Among the inflammatory dermatoses, acute contact dermatitis and psoriasis are the two skin diseases that benefit most from being examined with the help of LSM (Swindells et al, 2004; Hicks et al, 2003; Astner et al, JAAD, 2006; Astner et al, Dermatitis, 2006; Ardigo et al, 2009; Koller et al, 2009; Sauermann et al, 2002). Here, the characteristic features of spongiosis and vesicle formation have been described. The former appears as a bright accent in intercellular spaces of the epidermis, while the latter as sharply demarcated, partly chambered dark cavities with small or large lumen. Furthermore, reactive or accompanying inflammatory processes within the context of wound healing, tumors or in the context of infection (Meyer et al, 2005; Ulrich et al, 2008; Gonzalez et al, 2002; Goldgeier et al, 1999; Koller et al, 2009;
Longo et al, Arch Dermatol, 2005) or inflammations of autoimmune diseases were studied as well (Ardigo et al, BJD, 2007).

The inflammatory infiltrate in itself appears as a more or less heavily refractile, round to oval cells 8-10 μm in size, which, depending on the disease process in the epidermis, may be junctional, peripapillary, perivascular or diffuse in the superficial dermis. It was also possible to depict with LSM accompanying changes, e.g. of the connective tissue in autoimmune processes (Ardigo et al, BJD, 2007). Here, too, the evaluation is limited by the optical penetration depth and an accurate differentiation of various immune cells is not yet possible.

Finally, there are several reports on the study and diagnosis of cutaneous pigmentation disorders such as vitiligo and melasma (Ardigo et al, 2007; Kang HY et al, Exp Dermatol, 2010; Kang et al, JAAD, 2010; Lai et al, Skin Res Technol, 2011).

4.6 Other Indications

Confocal laser microscopy is also suitable for pathogen diagnosis. Fungal infections of the skin or the nail bed can be diagnosed directly at the patient without tissue processing. Hyphae and spores are represented as light-reflecting structures with typical morphology. (Meyer et al 2005) Mites such as Sarcoptes scabiei (itch mites) or Demodex folliculorum (face mites) are clearly identifiable, which is why this method is suitable for the rapid diagnosis of scabies or the detection and quantification of Demodex colonization in case of rosacea (Longo et al, Arch Dermatol, 2005; Levi et al, Lasers Med Sci, 2011). Bacterial and viral infections can be detected only indirectly through the morphology of the inflammatory response. The resolution is insufficient to show the pathogen. In herpes virus infections, typical acantholytic intraepidermal blisters are visible. Bacterial infections are associated with accumulations of neutrophils (Goldgeier et al, 2002; Gonzalez et al, J Clin Pathol, 1999).

In the field of cosmetology research, confocal laser microscopy for objectification and quantification of treatment effects is used. Skin aging is accompanied by a flattening of the dermoeipidermal interface. This can be quantified using a confocal laser microscope using the density of papillae tips in the horizontal section. For example, effects of UV radiation and antioxidants can be examined (Sauermann et al, BMC Dermatol, 2002; Yamashita et al, Exp Dermatol, 2007; Ulrich et al, BJD, 2009; Middelkamp-Hup et al, JID, 2006).
4.7 Fluorescence diagnostics in vivo

Fluorescence diagnosis is possible with the multi-wave microscopes. Only a few fluorescent dyes are approved for in vivo fluorescence diagnostics. These are especially used intravenously in ophthalmic diagnostic tests. Sodium fluorescein, after being excited with the wavelengths of 445 or 488 nm, exhibits a strong green fluorescence. Indocyanine green is excited at 785 nm and fluoresces in the near infrared range. Methylene blue and patent blue are used for in vivo marking of sinus tracts and sentinel diagnostics. These are red fluorescent dyes excited at 658 nm. The dyes can be topically applied to the skin surface and are sometimes still evident in the folds of skin and adnexa many hours and days later. They can be mixed with emollients and provide information about penetration routes and protection functions of topically applied substances (Suihko et al, 2005; Swindle L et al, JID, 2003; Teichmann et al, 2007; Lange-Asschenfeldt et al, J Biomed Opt, 2009). Sodium fluorescein can also be injected intradermally, indocyanine green and blue dyes, however, should not due to toxic effects and the risk of a permanent tattoo. Systematic studies of in vivo fluorescence diagnostics are pending.

5 Ex vivo confocal laser microscopy

The resolution and layer thickness of ex vivo confocal laser microscopy correspond to the in vivo technique (see 4.1). The maximum depth of penetration into the tissue in this case is approximately 0.05 mm so that only the respective outer sides of the tissue sections on the slide can be examined. Accordingly, the current main application of ex vivo confocal laser microscopy consists of examining the resection margins in tumor surgery using micro-graphically controlled surgical procedures (Käb et al, 2009; Patel et al, 2007; Schüle et al, 2009; Ziefle et al, 2010).

5.1 Examination technique

The examination can be done in reflective mode at 830 nm and in fluorescence mode at 445 and 658 nm (see below). When using the currently available VivaScope 2500 system, the prepared tissue sections are placed on the slide with some 0.9% NaCl solution and fixed in place with a clamp. The exam can commence after setting the best focus depth for clear and
crisp imaging. Analog to the in vivo process, scan fields sized 750 μm x 750 μm are used, which can be compiled with the device software to fields of any size (maximum is 14 mm x 14 mm). Larger tissue samples can currently only be studied using the manual shifting of the tissue on the slide. The saved fields can then be reviewed on the monitor either in an overview or with a zoom tool, and the scan fields can also be displayed individually at high resolution. A higher resolution with scan fields of 500 μm x 500 μm can be set in case of special issues.

5.1.1 Reflective mode

As with the in vivo mode, the structures in skin serve as a contrast agent. In ex vivo mode, these are in particular melanin, cellular structures, especially the nuclei, but also collagen and elastic fibers. For contrast enhancement by protein precipitation (better reflection), the native tissue is soaked in 10% acetic acid for approx. 1 minute and then rinsed off with NaCl solution. No further tissue preparation is required for reflective mode; the tissue is then examined natively as described.

5.1.2 Fluorescence mode

In fluorescence mode, the wavelengths 445 nm and 658 nm are available for the excitation of the fluorescent dyes introduced into the tissue; the excitation wavelength is here automatically masked by a filter system in the device so that only the fluorescent light is used for imaging. The objective is to use binding of the dyes to specific structures that is as selective as possible (e.g. cell nuclei for tumor diagnostics) to increase the contrast and evaluability. For example, acridine orange (Karen et al, 2009) can be considered for the blue wavelength of 445 nm, which makes the cell nuclei more prominent. In red mode at 658 nm, currently used fluorescent dyes are especially toluidine blue or methylene blue, which is still largely an experimental diagnostic technology that is not yet standardized.

In principle, after prior contrast enhancement by protein precipitation, as described above, fluorescence technique requires that the tissue is placed in the fluorescent dyes so that the color can diffuse into the uppermost layers of tissue and subsequently can be used for fluorescence imaging. There are currently no standardized examination techniques for this that may be specified in the guideline. The examination and evaluation of additional dyes can be expected in the near future.
5.2 Indications

The only indications for ex vivo confocal microscopy that can currently be specified are the examinations of edge incisions within the scope of a micrographically controlled surgery for tumor excisions and frozen section techniques. The biggest advantage of this method - compared to the previous excisional margin-controlled processes - would be an extremely short examination time (approximately 5 minutes per image).

Preliminary studies to evaluate the excisional margin control with the confocal microscope are so far available only for the reflective mode. Schüle et al describe 2009 with nearly 250 excisional margin images a sensitivity of the analysis technique between 0% and 94% and a specificity between 30% and 100%, depending on the excision technique (centered incisions, marginal incisions, "muffin-can" incisions). In a second series of 312 images, Ziefle et al (2010) concluded sensitivity between 73% and 94% and specificity between 36% and 78%. In our own studies, (Käb et al, 2009), we examined 134 margin and depth specimens and found a specificity of 90% and a sensitivity of 82%. The listed data show that the method currently cannot entirely replace hematoxylin-eosin stain histology in micrographically controlled surgery. With clearly positive resection margins examined with confocal laser scanning microscopy, however, the indication for a re-excision could be established quickly so that treatment can be accelerated significantly.

Fluorescence diagnostics is a promising additional approach. With the use of acridine orange as a dye, which allows tumor cells to emerge in high contrast, Karen et al (2009) used 149 confocal images to reach a specificity of 89.2% and a sensitivity of 96.6%, which makes this method highly interesting for clinical use. Another approach could be the coupling of fluorescent dyes with specific antibodies (for example, Ber-EP4 for basal cell carcinoma).
6 Literature


The guideline was adopted by the 2+2 Commission without objections.

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