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Presurgical mapping of basal cell carcinoma or squamous cell carcinoma by confocal laser endomicroscopy compared to traditional micrographic surgery: a single-centre prospective feasibility study

Background: At present, no ideal diagnostic tools exist in the market to excise cancer tissue with the required safety margins and to achieve optimal aesthetic results using tissue-conserving techniques. *Objectives*: In this prospective study, confocal laser endomicroscopy (CLE) and the traditional gold standard of magnifying glasses (MG) were compared regarding the boundaries of in vivo basal cell carcinoma and squamous cell carcinoma. *Materials & methods*: Tumour diameters defined by both methods were measured and compared with those determined by histopathological examination. Nineteen patients were included in the study. *Results*: The CLE technique was found to be superior to excisional margins based on MG only. Re-excision was required in 68% of the cases following excision based on MG evaluation, but only in 27% of the cases for whom excision margins were based on CLE. *Conclusion*: Our results are promising regarding the distinction between tumour and healthy surrounding tissue, and indicate that presurgical mapping of basal cell carcinoma and squamous cell carcinoma is possible. The tool itself should be developed further with special attention to early detection of skin cancer.

Key words: confocal laser endomicroscopy, magnifying glass, basal cell carcinoma, squamous cell carcinoma

Skin cancer presently poses a considerable public health threat of global importance. Patients from all ethnic, socio-economic, and demographic groups suffer from skin cancer with increasing frequency [1, 2]. Therefore, it is one of the most common cancers affecting humans today [3, 4]. According to American Cancer Society data and the Skin Cancer Foundation Statistics, skin cancer comprises one third of newly diagnosed cancer disease worldwide. The two most common subtypes of non-melanocytic skin cancer (NMSC) are basal cell carcinoma (BCC) (80%) and squamous cell carcinoma (SCC) (16%) [2-5]. The probability of skin cancer rises as patient age increases. Against a background of demographic change, with the average age rising in western society for the next 30 years, a doubling of the incidence of NMSC is forecast [6].

As UV dose is one of the primary reasons for the occurrence of BCC and SCC, sun exposed areas, such as the face, scalp, lips, forearms, and hands, are frequently affected [6]. Surgical excision of cancer tissue in these exposed areas clearly demands an optimal aesthetic outcome. Therefore, preservation of healthy tissue with respect to the aesthetic entities of the face is the central issue. The surgeon has to strike the right balance between complete excision of cancer tissue and preservation of the natural body shape [6]. Recently, extensive research was conducted in order to evaluate the most suitable tool for accurate skin cancer excision in NMSC [7, 8]. Hence, Mohs micrographic surgery, a step-by-step surgical excision, is currently the gold standard for the treatment of NMSC and provides the best results with respect to cure and recurrence rates [7, 9, 10]. Nevertheless, current surgical tools still reveal evidence of considerable weaknesses regarding efficiency and selectivity, and therefore produce poor results with a high risk of follow-up resection [9, 10].

CLE was initially developed for cavity diagnostics in gastroenterology. Recently, numerous international groups have evaluated its application in various surgical fields with promising results [11-13]. Because the laser endomicroscope allows visualisation of cancer cells up to the cellular level, this instrument seems to be well suited for the diagnosis of cancer cells of the skin.

The key aim of the present study was to determine whether CLE is a suitable technology for the detection and evaluation of lesions suspected to be skin cancer. Furthermore, we investigated whether CLE was superior to the technique of micrographic surgery based on MG.

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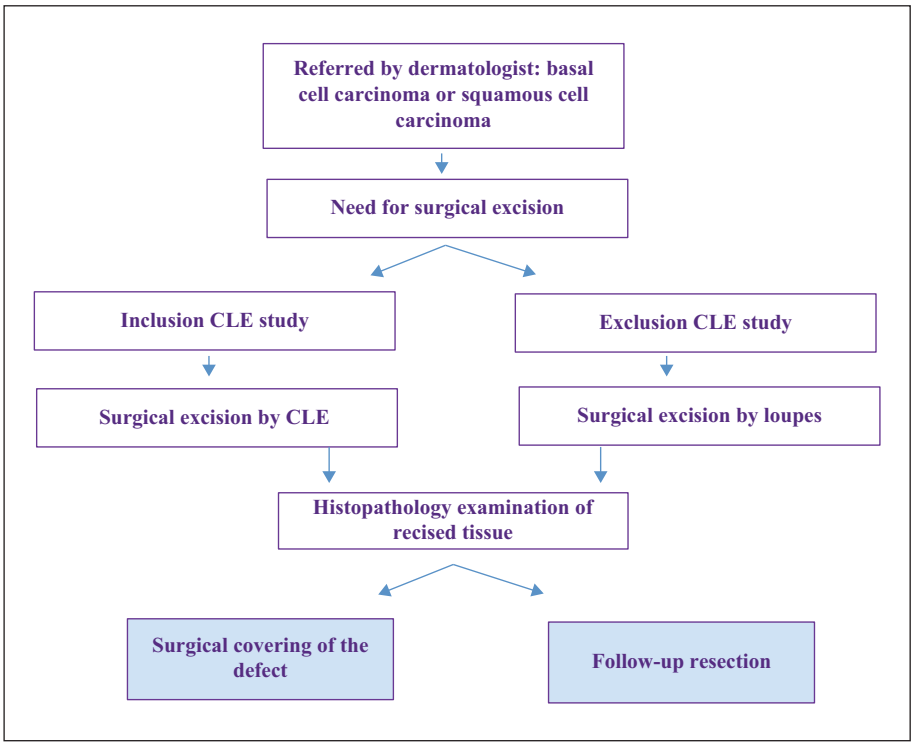

Figure 1. Flow diagram of treatment decisions.

Methods

The single-centre, prospective study was conducted at the Department of Plastic Surgery, Faculty of Medicine, University of Witten/Herdecke, Campus Cologne-Merheim. The study was reviewed and approved by the Ethical Review Committee of the University of Witten Herdecke, Germany (votum nr. 99/2014). Complete informed consent was obtained from all patients.

Materials

CystoFlex systems (Cellvizio® (Mauna Kea Technologies, Paris, France) are intended for intraoperative non-invasive *in vivo* histological imaging of cellular, sub-cellular, and even sub-nuclear structures of the internal microstructure of tissues in anatomical tracts with high resolution [13]. They are composed of a laser scan unit (LSU-488) and an imaging mini-probe (ProFlex). The laser system has an excited wavelength of 488 nm and a detector for 500-650 nmA [14]. A miniaturized scanner, 2.3 mm in diameter, is integrated into the tip of a conventional endoscope. The image of the examined surface and subsurface is transferred onto a screen in real time [15, 16]. The field of view is 240 × 200 μm. Structures up to 150 microns in depth can be visualised. *In vivo* histology is possible with nearly 1,000-fold magnification [17].

Patient selection

Each patient who was referred to our department by practicing dermatologists with the diagnosis of BCC or SCC of the skin, and requested surgical excision, was offered surgical excision assisted by CLE (*figure 1*). Nineteen patients were enroled (10 patients with BCC and nine patients with SCC) (*table 1*, *figure 2*). Inclusion criteria were as follows: (a) participants of either gender must be at least 18 years old and in a good physical condition, (b) diagnosis of BCC or SCC of the skin by the practicing dermatologist, (c) surgical excision of the skin cancer is requested, and (d) patients agree to treatment with CLE. Exclusion criteria included: (a) lack of consent and compliance to participate in the study, (b) acute instability, (c) pregnancy or nursing, and (d) prior excision of skin cancer at the same location.

Surgical procedures

All operations were performed under local anaesthesia. Patients were prepared and prepped for the operation in the customary fashion. All enroled patients were evaluated by all three methods; MG, CLE, and histopathological examination.

Skin cancer boundaries were marked circumferentially first by inspection only, and second by using MG at four-fold magnification as our current standard of care. Afterwards, the drawing was removed and a second independent surgeon marked the boundaries visualised by CLE. During the imaging of the tissue, the tip of the confocal miniprobe was gently but firmly positioned onto the skin [18]. Finally, the cancer was excised with the addition of a safety margin following current guidelines. Later, the excised dermal tissue was sent for histopathological examination (*figures 3*, *4*).

The diameters of the ovals of the skin cancers were measured clockwise, between the 3 and 9 o'clock positions and between the 6 and 12 o'clock positions separately after

Table 1. Demographic, clinical and tumour characteristics of the 19 enroled patients.

Patient no.	Age	Gender	Type of tumour	Area	Months since first awareness
1	77	f	basal cell carcinoma	face	5
2	89	m	squamous cell carcinoma	scalp	120
3	89	m	squamous cell carcinoma	scalp	120
4	80	f	basal cell carcinoma	chin	120
5	88	f	squamous cell carcinoma	forearm	12
6	86	m	squamous cell carcinoma	scalp	36
7	75	f	basal cell carcinoma	scalp	120
8	71	f	basal cell carcinoma	scalp	18
9	81	f	basal cell carcinoma	lower lid	12
10	62	m	squamous cell carcinoma	base of the nose	24
11	80	m	basal cell carcinoma	face	48
12	92	m	squamous cell carcinoma	shoulder	12
13	80	m	basal cell carcinoma	scalp	24
14	92	f	squamous cell carcinoma	forearm	36
15	92	f	squamous cell carcinoma	upper arm	36
16	89	m	basal cell carcinoma	face	48
17	89	m	basal cell carcinoma	face	48
18	89	m	squamous cell carcinoma	back	48
19	82	f	basal cell carcinoma	scalp	36

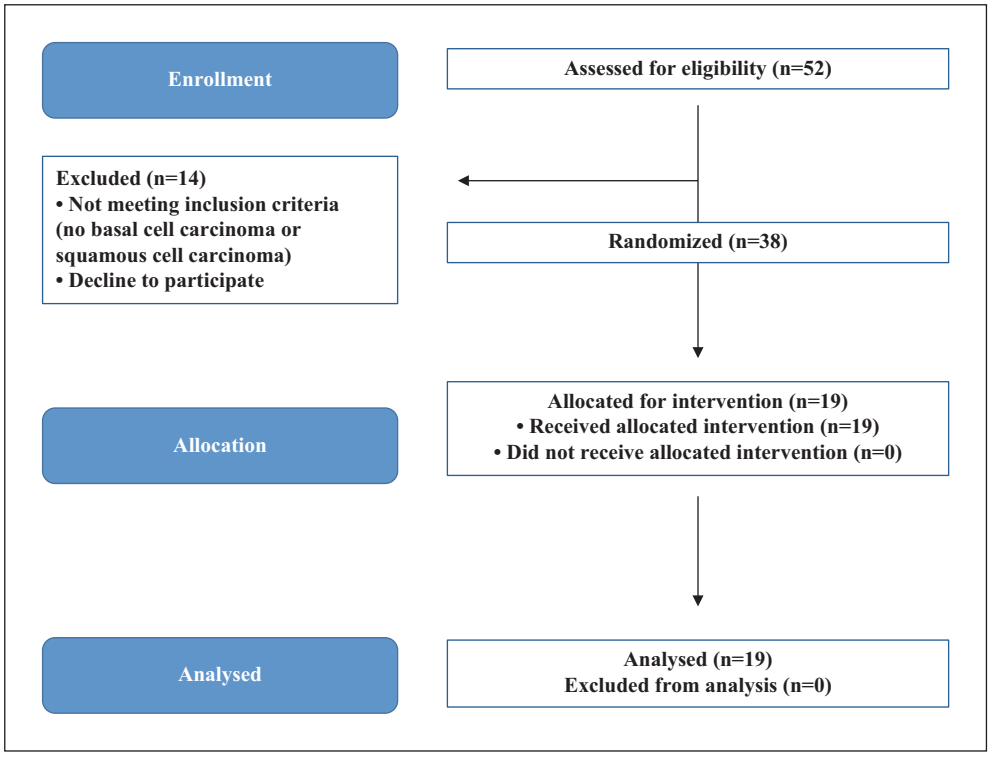

Figure 2. Flow diagram of current study.

CLE, MG and histopathological examination. Based on these diameters, the surface area was calculated:

skin cancer area = [diameter between 3 and 9 o'clock] /2

+ [diameter between 6 and 12 o'clock] /2×pi [~ 3.14]

Further therapy followed the SOC of the Cologne Merheim Department of Plastic Surgery. All patients responded well after CLE. No dropouts or adverse events were noted throughout the entire study period (*figure 2*). The cancer tissue was examined by an independent pathologist who was blinded to patient participation in the current study.

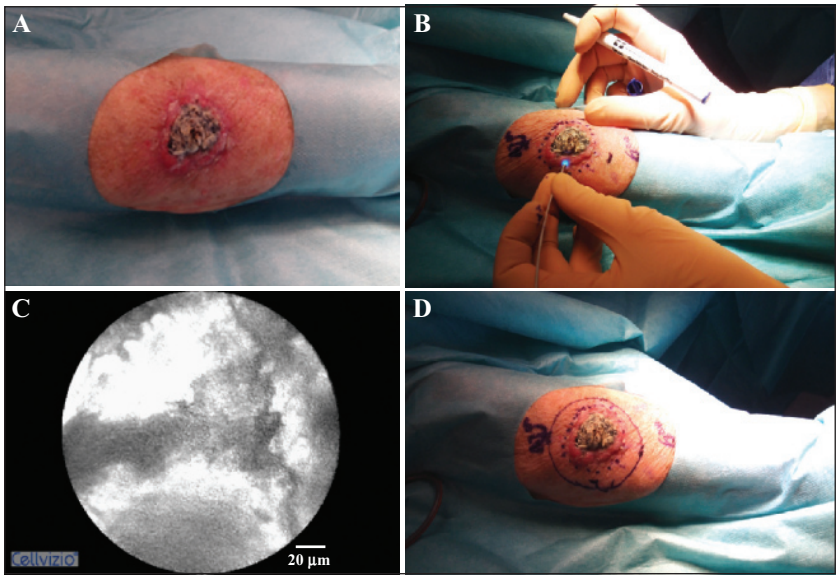

Figure 3. Patient 2: **(A)** squamous cell carcinoma prior to surgery; **(B)** drawing the resection edges using confocal laser endomicroscopy; **(C)** monitor output from confocal laser endomicroscopy; and **(D)** resection surface area based on CLE >MG.

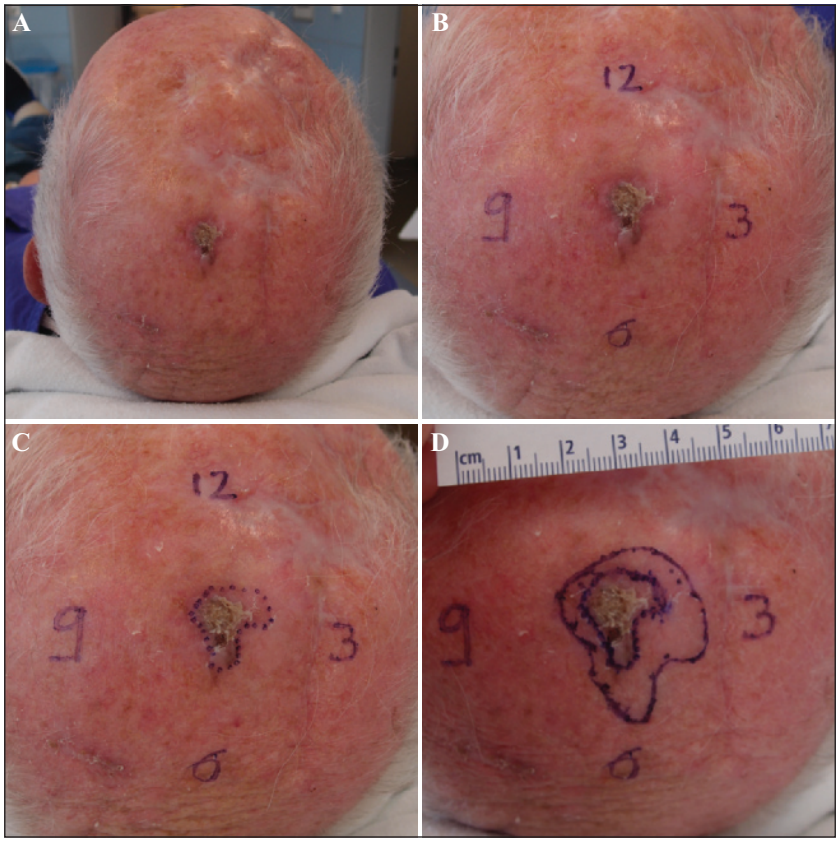

Figure 4. Patient 4: **(A)** basal cell carcinoma in the high-parietal region prior to surgery; **(B)** marking clockwise; **(C)** drawing of resection edges based on magnifying glasses; and **(D)** drawing of resection edges based on confocal laser endomicroscopy.

Statistical analysis

We used Microsoft Excel (2013, Microsoft, USA) to manage data and design the charts. Data were collected prospectively and checked for completeness and accuracy prior to analysis. The final analysis was performed with SPSS (IBM, USA) version 21. All three paired samples were analysed for statistically significant differences first by the Friedman test. In the case of significant differences, we used the Wilcoxon test for pair-wise comparisons. Statistical significance was accepted at p values <0.05.

Table 2. Pairwise comparison between areas evaluated by MG, CLE, histopathological examination, and visual inspection with respect to mean tumour surface area; descriptive statistics and Wilcoxon rank sum test for paired data (significant data marked).

Wilcoxon Signed Ranks Test - Surface area measured						
	Inspection - Histopathology	MG - Histopathology	CLE - Histopathology	MG - Inspection	CLE - Inspection	CLE - MG
Z	-3.380[b]	-1.851[b]	-1.154[c]	-1.886[c]	-3.783[c]	-2.616
Asymp. Sig. (2-tailed)	.001	.064	.248	.059	.000	.009
b. Based on positive ranks c. Based on negative ranks						
Descriptive statistics	**N**	**Minimum**	**Maximum**	**Mean**		**Std. Deviation**
Surface area measured using						
Histopathology	19	.50	11.00	3.4942		2.92650
Inspection	19	.06	7.07	2.1425		2.24186
MG	19	.06	9.88	2.5166		2.89685
CLE	19	.71	8.95	3.7306		2.95438

Results

Research group characteristics

Among all patients assessed for eligibility, 19 patients (nine females and 10 males) were enroled in the current study and completed the trial (four patients declined to participate in the study and ten patients did not meet the inclusion criteria). Study data were found to be complete for all enroled patients (*figure 2*). All participants were strictly examined as required by the case report form during outpatient treatment.

Patients' ages ranged from 62 to 92 years (mean: 83 years). Patients were referred for excision of basal cell carcinoma ($n = 1$) and squamous cell carcinoma ($n = 9$) (excision was performed, on average, 48 months from the first awareness of cancer) (*table 1*). Health and safety reasons were mentioned as motivation for surgery for most patients. The majority (58%) considered aesthetic concerns to be very important. All patients with only facial skin cancer reported a good aesthetic outcome as a central issue of their treatment.

Preciseness of cancer boundaries and surface area

The margin between cancer tissue and healthy tissue was found to be difficult to evaluate based on both inspection and MG. Using the Friedman test, we found statistically significant differences ($p<0.001$) between tumour surface areas evaluated, based on visual inspection, MG, CLE and histopathological examination.

The mean tumour surface area evaluated, based on visual inspection, was clearly smaller than that determined by histopathological boundaries (2.14 cm^2 versus 3.49 cm^2; $p = 0.001$). Using MG, we confirmed *in sano* excision of the skin cancer in only 32% of the cases. We found no significant differences in cancer surface areas as measured by MG versus visual inspection or histopathological examination (*table 2*).

CLE complete excision of cancerous tissue, involving a single operation, was achieved in 63% of all cases. A comparison of both groups using side-by-side box plots illustrated that the CLE-based surface areas were closer in size to the histopathological-based areas than the MG-based areas (*figure 5*). Furthermore, all results based on CLE deviated only slightly from those based on histopathological results, in contrast to those based on MG. The box plot diagram revealed that surface areas based on CLE tended to be greater than necessary, compared to histopathological evaluations; in contrast, the surface areas based on MG were usually too small relative to histopathological evaluations (*table 2*). We found no statistically significant difference between mean tumour surface area based on CLE or histopathological examination (3.73 mm^2 versus 3.49 mm^2, $p = 0.248$). Clear differences were found for mean surface area based on CLE and visual inspection (2.15 cm^2 versus 3.73 cm^2; $p<0.001$) and CLE and MG (2.52 cm^2 versus 3.73 cm^2; $p = 0.009$) (*table 2*). The Wilcoxon rank test showed that in 15 cases, the tumour surface area based on CLE was greater than that based on MG. In four cases, the surface area based on MG was greater.

Discussion

According to the literature, we found poor results for precise visualisation of cancer boundaries of basal cell carcinoma and squamous cell carcinoma based on MG [9, 10]. Of these patients, 68% would have required re-excision of their skin cancer. Micrographic surgery is known to be limited by the low level of magnification, blindness for basal excision of cancer tissue, lack of accuracy, and experience of the surgeon. Repeated surgical interventions cause emotional and physical stress for mainly elderly patients and also raise treatment costs [19].

Due to these unsatisfactory results, extensive research was conducted in order to improve accurate skin cancer excision for NMSC. In the scientific literature, there are various studies of CLE-assisted *in vivo* imaging of the internal microstructure of tissues in anatomical tracts, with convincing results [11, 17, 20]. As a new field of application for CLE, excellent results have been demonstrated for brain cancer cells and the detection of metastases. Today, it is widely accepted that with the use of CLE technology, the

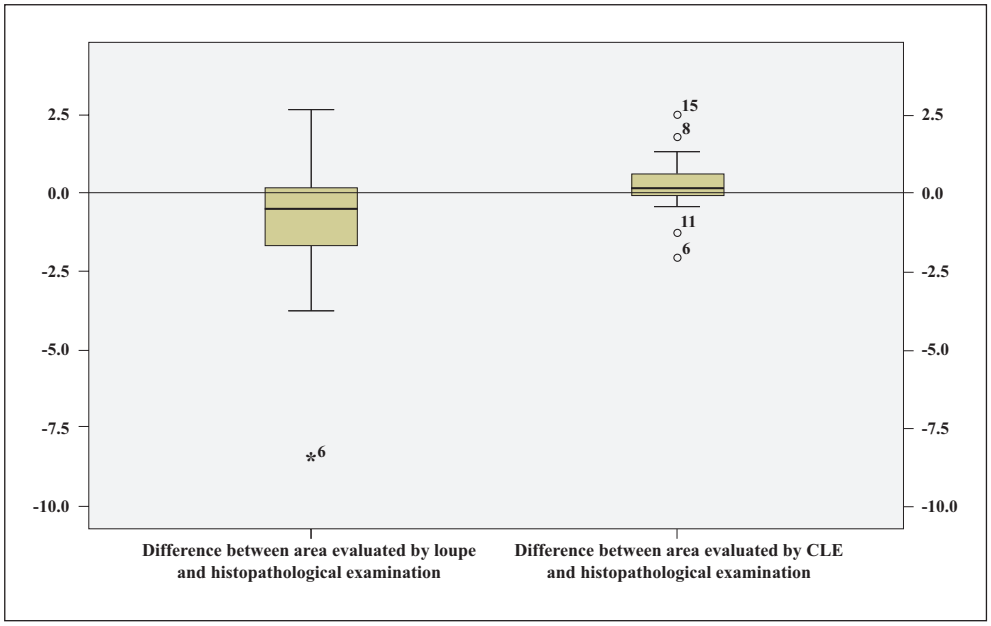

Figure 5. Box plots depicting differences between lesion surface areas according to method used to determine lesion boundaries. Left: MG-determined area minus histopathology-determined area. Right: CLE-determined area minus histopathology determined area.

number of intraoperative biopsies and the need for removal of non-neoplastic structures can be reduced drastically in these fields [21, 22].

Investigation of human skin using CLE has been reported, including microcirculation, histomorphology, and burn wound assessment, with promising results [23-25]. Furthermore, the application of *in vivo* confocal laser scanning microscopy has been analysed for different skin diseases, such as pemphigus foliaceus and the differentiation of its subtype [26]. In our current study, we focused on BCC and SCC of the skin which are difficult to diagnose at an early stage with common instruments [27]. Carlos *et al*. found confocal scanning laser microscopy (CSLM) suitable for the evaluation of superficial pigmented BCC [28]. In dermatological research, vascular patterns of non-pigmented tumoural skin lesions were found to be recently clearly visualised by *in vivo* reflectance confocal microscopy [29]. Both epidermal and dermal structures were shown to be visualised by CLE, equivalent to histological examination results [30, 31]. González and Tannous found that *in vivo* confocal reflectance microscopy facilitates the diagnosis of BCC [32]. Further research groups found promising results for the detection of up to 100% and for distinguishing BCC from surrounding tissue on the basis of highly specific criteria [33-36]. Flores *et al*. even reported positive results for the detection of residual intraoperative NMSC using CLE [36]. For SCC, several research groups have identified characteristic key features which could be clearly distinguished from other skin anomalies [37].

In our present prospective study, we could confirm the reported findings regarding accurate detection of cancerous tissue by CLE. We found that the mean cancer surface determined by CLE was clearly greater than that determined by MG, and that both groups differed significantly. Cancer surface areas determined by CLE were generally found to be larger than the surface area of the cancer defined by histopathological evaluation. In our study, in 63% of all cases, skin cancer was excised completely based on CLE. In 27% of the cases, the margins were too close, and therefore re-excision of the skin cancer was required.

In addition, we evaluated whether excision margins were closer to the cancer surface area based on MG or CLE than on histopathological evaluation. To answer this question, we calculated the deviation of cancer surfaces following CLE, MG, and histopathological evaluation. As illustrated by box plots, CLE was clearly superior to MG in our study population. The box plot of CLE/histopathological differences was narrower than the MG/histopathological differences, thus the CLE-based areas were closer in size to the histologically-determined areas (*figure 5*).

In summary, we demonstrate, in this initial study, that CLE enables the visualisation of the structure of the healthy dermis and its transition to an inhomogeneous arrangement, on a cellular basis, in BCC and SCC. We found that cancer cells provide good signal intensity and adequate contrast. High density and interconnectedness of dermal cells could be clearly identified and were distinguishable from surrounding healthy skin with low density (*figure 6*). Different settings of the image software (ImageCell, Mauna Kea Technologies, France) enabled us to optimise the graphic output (*figure 7*). For the visualisation of skin cancer, we found CLE to be superior to micrographic surgery using MG.

Study limitations

We recommend performing an extension of the present study to include more patients. Collagen and elastin fibres are components of the human skin that cause autofluorescence emission [38]. Furthermore, hypertrophic or hyperkeratotic lesions are difficult to distinguish early on [30]. Auto-fluorescence itself may reduce accuracy of CLE. Therefore, this methodology should be developed further with special attention to the detection of skin cancers.

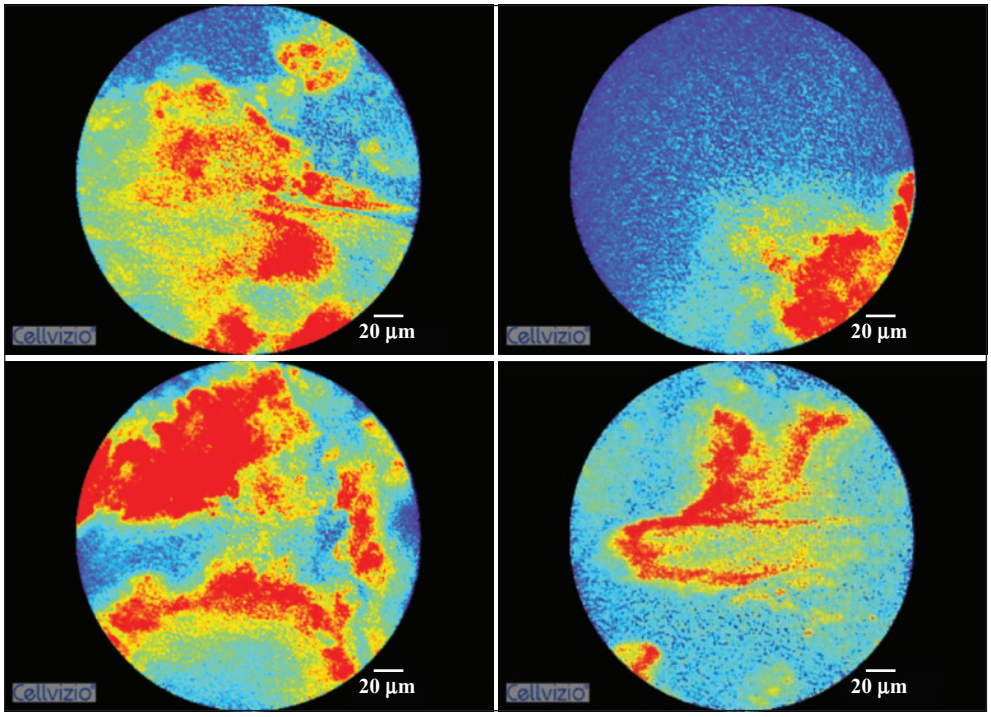

Figure 6. Monitor output confocal laser endomicroscopy: tumour tissue of various densities, with high discriminatory power for healthy skin (blue) and tumour tissue (red).

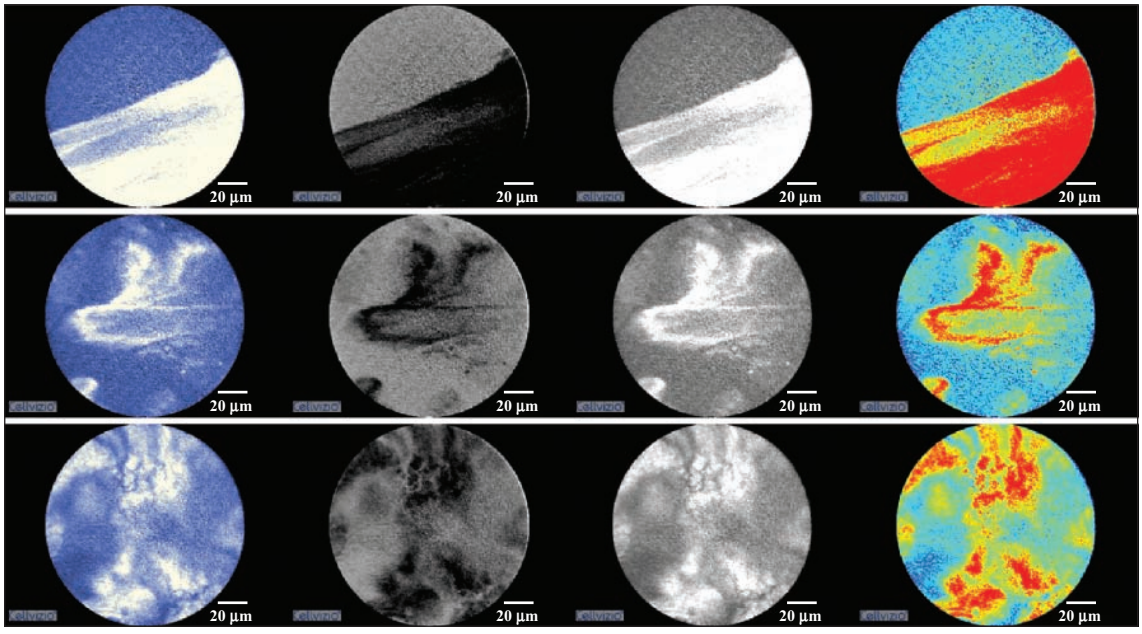

Figure 7. Various monitor outputs provided by the Cellvizio® system for the same tumour entity.

Conclusion

In the hands of an experienced user, CLE allows accurate intraoperative visualisation of cancer margins and cancer-free tissue. We envisage that the development of an intelligent diagnostic device based on CLE technology will greatly improve the sensitivity and specificity of screening tests for skin cancer prevention. This may provide adequate adjuvant therapy at a very early stage and guarantee an optimal aesthetic outcome, particularly with respect to the natural body shape. Based on our findings, we believe that CLE has clear potential for intraoperative visualisation of skin cancer. Further development could enable CLE to play a central role in *in vivo* screening diagnosis in the future. ■

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the trial results. Cellvizio® was not involved in the study design, nor in the collection or analysis or interpretation of the data, which were performed by impartial professionals.

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