Year : 2022

Ex vivo confocal microscopy for surgical margin assessment: a histology compared study on 109 specimens.

Grizzetti Lorenzo

Grizzetti Lorenzo, 2022, Ex vivo confocal microscopy for surgical margin assessment: a histology compared study on 109 specimens.

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive http://serval.unil.ch
Document URN : urn:nbn:ch:serval-BIB_4611B034FBF74

Droits d’auteur
L'Université de Lausanne attire expressément l'attention des utilisateurs sur le fait que tous les documents publiés dans l'Archive SERVAL sont protégés par le droit d'auteur, conformément à la loi fédérale sur le droit d'auteur et les droits voisins (LDA). A ce titre, il est indispensable d'obtenir le consentement préalable de l'auteur et/ou de l'éditeur avant toute utilisation d'une oeuvre ou d'une partie d'une oeuvre ne relevant pas d'une utilisation à des fins personnelles au sens de la LDA (art. 19, al. 1 lettre a). A défaut, tout contrevenant s'expose aux sanctions prévues par cette loi. Nous déclinons toute responsabilité en la matière.

Copyright
The University of Lausanne expressly draws the attention of users to the fact that all documents published in the SERVAL Archive are protected by copyright in accordance with federal law on copyright and similar rights (LDA). Accordingly it is indispensable to obtain prior consent from the author and/or publisher before any use of a work or part of a work for purposes other than personal use within the meaning of LDA (art. 19, para. 1 letter a). Failure to do so will expose offenders to the sanctions laid down by this law. We accept no liability in this respect.
Ex vivo confocal microscopy for surgical margin assessment: a histology-compared study on 109 specimens.

THESE

préparée sous la direction du Docteur François Kuonen

et présentée à la Faculté de biologie et de médecine de l’Université de Lausanne pour l’obtention du grade de

DOCTEUR EN MEDECINE

par

Lorenzo GRIZZETTI

Médecin diplômé en Italie
Originaire d’Italie

Lausanne
2022
Vu le rapport présenté par le jury d'examen, composé de

Directeur.trice de thèse
Dr François Kuonen

Co-Directeur.trice de thèse
Prof. Emmanuella Guenova

Expert.e
Prof. John Prior

Vice-Directeur de l'Ecole doctorale

la Commission MD de l'Ecole doctorale autorise l'impression de la thèse de

Monsieur Lorenzo Grizzetti

intitulée

Ex vivo confocal microscopy for surgical margin assessment: a histology compared study on 109 specimens

Lausanne, le 29 septembre 2022

pour Le Doyen
de la Faculté de Biologie et de Médecine

Monsieur le Professeur John Prior
Vice-Directeur de l'Ecole doctorale
La microscopie confocale ex-vivo pour l’analyse des marges chirurgicales : une étude de comparaison histologique sur 109 spécimens

Background

Afin de prévenir la récidive locale ou la dissémination à distance du cancer de la peau, l’évaluation des marges chirurgicales est fondamentale. L’examen histologique des spécimens inclus en paraffine ou cryogénisés et après préparés en lames colorées avec hématoxyline et éosine, représente actuellement le « gold-standard » pour l’évaluation des marges, mais il s’agit d’une procédure chronophage. La microscopie confocale à balayage laser (CLSM) ex-vivo est une nouvelle technologie qui permet d’analyser rapidement le tissu frais, non fixé, en permettant d’évaluer vite en périopératoire les marges chirurgicales. Des études ont déjà souligné que cette technique a une haute sensibilité et spécificité pour l’analyse des marges chirurgicales de carcinome basocellulaire.

Objective de l’étude

Dans notre étude nous avons évalué l’efficacité d’une nouvelle microscopie confocale ex-vivo pour l’évaluation périopératoire des marges chirurgicales. Nous avons aussi comparé la sensibilité et la spécificité de cette technique pour les différents types de tumeur et pour les différents types de spécimen chirurgical.

Méthodes

Entre janvier et mars 2021, nous avons analysé de façon prospective 109 échantillons dont 16 biopsies et 93 spécimens chirurgicales de carcinome basocellulaire et épidermoïde avec CLSM ex-vivo en utilisant Histolog Scanner V2. Les échantillons chirurgicaux comprennent des excisions fusiformes, slow-Mohs périphériques e du compartiment profond et excisions Mohs. Nous avons enregistré le temps nécessaire pour analyser l’image après l’excision chirurgicale et nous avons évalué la qualité des images obtenues pour chaque spécimen analysé.

La présence ou l’absence de tumeur a été estimée sur la base des images obtenues à travers la CLSM ex vivo et a été comparée avec l’examen histologique conventionnel des spécimens inclus en paraffine ou cryogénisées (Mohs) et après préparés en lames colorées avec hématoxyline et éosine.

Résultats

Le temps moyen pour traiter les spécimens a été de 5.1±3.4 minutes en utilisant le microscope Histolog Scanner. Nous avons obtenu le 89% des images de haute qualité. Le temps moyen pour analyser les images avec la microscopie confocale a été de 1 ± 0.76 minutes. La sensibilité et la spécificité diagnostique pour la CLSM ex-vivo comparée avec l’analyse standard en hématoxyline et éosine a été respectivement de 93% et de 100% si effectuée sur les biopsies tumorales. La sensibilité et la spécificité globale pour l’évaluation des marges chirurgicales en comparant la CLSM ex-vivo avec la technique classique hématoxyline et éosine était respectivement de 61,5% et de 95% avec variation qui dépendait du type de tumeur ou du type de spécimen chirurgical analysée. En particulier, nous avons obtenu le 80% de sensibilité et le 100% de spécificité pour l’analyse des marges de carcinome basocellulaire.

Conclusion

Nos données suggèrent que la microscopie confocale a balayage laser ex vivo en utilisant Histolog Scanner V2 pourrait être une aide valide pour les chirurgiens pour une analyse périopératoire vite et précise des marges chirurgicales.
Ex vivo confocal microscopy for surgical margin assessment: A histology-compared study on 109 specimens

L. Grizzetti  |  F. Kuonen

Department of Dermatology and Venereology, Hôpital de Beaumont, Lausanne University Hospital Center, Lausanne, Switzerland

Correspondence
François Kuonen, Department of Dermatology and Venereology, Hôpital de Beaumont, Lausanne University Hospital Center, CH-1011 Lausanne, Switzerland. Email: francois.kuonen@chuv.ch

[Correction added on 4-June-2022, after original publication: the ethics statement was added.]

Abstract
Background: The assessment of surgical margins is mandatory to prevent local recurrence or distant dissemination of skin cancers. Histological examination of haematoxylin and eosin (H&E)-stained slides from paraffin-embedded or frozen samples is the gold standard for margin assessment, but is a time-consuming procedure. Ex vivo confocal laser scanning microscopy (CLSM) is an upcoming technique that scans unfixed fresh tissue rapidly, allowing fast per-operative margin assessment.

Objective: Here, we propose to assess the efficiency of a new ex vivo confocal microscope for the per-operative assessment of surgical margins.

Methods: We analyzed 16 biopsies and 93 surgical specimens of basal cell and squamous cell carcinomas by ex vivo CLSM using Histolog® Scanner V2. Surgical specimens included fusiform excisions, slow-Mohs peripheral and deep compartments, and Mohs excisions. The time required from surgical excision to image analysis was recorded and the quality of the images obtained for each specimen assessed. The presence or absence of tumour was estimated based on ex vivo CLSM images and compared with conventional H&E-stained sections from paraffin-embedded or frozen (Mohs) specimens.

Results: Mean time for specimen processing using Histolog Scanner was 5.1 ± 3.4 min. We obtained 89% of high quality images. Mean time for confocal image analysis was 1 ± 0.76 min. The diagnostic sensitivity and specificity for ex vivo CLSM compared to classical H&E procedures were respectively 93% and 100% when performed on tumour biopsies. The overall sensitivity and specificity for ex vivo CLSM for margin assessment compared to classical H&E procedures were respectively 61.5% and 95%, with variations depending on the type of tumour or surgical specimen analyzed. In particular, we obtained 80% sensitivity and 100% specificity for the assessment of BCC surgical margins.

Conclusion: Our data suggest that ex vivo CLSM using Histolog® Scanner V2 could be a valid help for surgeons for a fast and accurate per-operative margin analysis.
1 | INTRODUCTION

Basal (BCC) and squamous (SCC) cell carcinoma are the most frequent cancers in human, for which the gold standard therapy is surgical removal with histopathological analysis of the surgical margins to assess complete excision.\(^1,2\) For high-risk tumours, three-dimensional micrographic analysis (Mohs micrographic or slow-Mohs micrographic techniques) is required to reduce the risk of relapse.\(^3\) While conventional histopathological analyzes assess 1%–2% of the surgical margins, micrographic analyzes are expensive, time-consuming and unfortunately not widely available procedures.

In the last years, ex vivo confocal laser scanning microscopy (CLSM) has developed as an optical method allowing high-resolution images of fresh, unfixed tumour tissue specimens like BCC, SCC, dermatofibrosarcoma and other adnexal tumours.\(^4\) Ex vivo CLSM allows fast analysis, sparing the time-consuming and costly procedure of tissue fixation, cutting and staining. In addition, it allows complete analysis of the surgical margins. The diagnostic accuracy of ex vivo CLSM compared to histopathological analyzes has been evaluated in numerous studies, mostly for BCC,\(^5\)\(^\text{--}\)\(^11\) although with varying sensitivity (73%–100%) and specificity (89%–100%) rates. Horn et al reported high sensitivity (95%) and specificity (96%) for SCC too.\(^12\)

Here, we tested the novel, improved Histolog® Scanner V2 (Figure 1) for the ex vivo assessment of BCC and SCC biopsies and surgical margins in a clinical setting and workflow. To do so, we compared the confocal digital images to H&E-stained frozen (Mohs) or paraffin-embedded sections.

2 | MATERIAL AND METHODS

2.1 | Setting

Between January and March 2021, we prospectively analyzed 109 specimens, of which 52 BCC (48.7%), 55 SCC (50.4%) and 2 basosquamous carcinomas (BSC; 1.8%) with Histolog® Scanner V2 (Figure 1) at Lausanne University Hospital. The study design was approved by the institutional review board of Lausanne University Hospital CHUV, and the local ethics committee (study 2015-00187). Each patient enrolled provided written informed consent. Inclusion criteria were age >18, clinically diagnosed BCC or SCC, for which surgery was indicated. Exclusion criteria was age <18. Histolog® Scanner V2 is a 488 nm fluorescence-based confocal laser scanning microscope with 2 µm lateral resolution, approximately 30 µm penetration depth and a 48 × 36 mm field of view, manufactured and provided by Samantree Medical SA, Switzerland. Images of the

What’s already known about this topic?

- Ex vivo fluorescence confocal laser scanning microscopy (CLSM) allows fast histopathological analysis of fresh tissues
- CLSM has a high sensitivity and specificity for the assessment of basal cell carcinoma surgical margins

What does this study add?

- Our study reports the diagnostic accuracy of a new ex vivo confocal microscope
- Furthermore, it compares the sensitivity and specificity of CLSM according to tumour types and surgical specimen

![Histolog® Scanner V2](image-url)
full field of view can be obtained either in a “preview”, low-resolution (15 s) or in an “acquisition”, high-resolution mode (1 min). LG and FK were the dermatosurgeons. LG and FK evaluated the confocal digital images. Before the start of the study, they both had a full-day instruction on how to use the Histolog® Scanner V2. Slow-Mohs and standard paraffin-embedded sections were evaluated by an independent dermatopathologist. FK is the Mohs surgeon who evaluated the H&E staining of Mohs frozen sections. The different types of tumours are shown in Table 1. Mean age of patients was 74.1 years old, with a range between 39 and 98 years. Percentage of male and female patients was 76.9% and 23.1% respectively. All BCC and SCC subtypes (including 2 BSC) were included.

2.2 Procedure

Before imaging on CLSM, slow-Mohs specimens were excised with a 2–3 mm margin, perpendicular to skin surface incision and prepared according to the “Tüebinger Torte” method, creating bases (deep margin) and lateral samples (peripheral margins).13 Deep margins of fusiform excisions were imaged without prior tissue dissection. Mohs samples were excised with a 45° angles. Debulking and flattening were performed as required for standard preparation for Mohs micrographic analysis. Altogether, we distinguished biopsies and surgical margin specimens, including: deep margins (from fusiform excisions and slow-Mohs bases), peripheral margins (slow-Mohs lateral surgical margins) and Mohs excisions. Importantly, obtaining adequate positioning of the specimen on the Histolog® dish, in order to get high quality images, was the most time-consuming part of the whole procedure.

Table 1: Frequency of different tumour type specimens

| Tumour type          | Subtype          | Number | %     |
|----------------------|------------------|--------|-------|
| BCC                  | Nodular          | 52     | 47.7  |
|                      | Micronodular     | 9      | 8.3   |
|                      | Infiltrative     | 22     | 20.2  |
|                      | Superficial      | 3      | 2.8   |
| SCC                  | Well differentiated | 55     | 50.5  |
|                      | Moderately differentiated | 20     | 18.3  |
|                      | Poorly differentiated | 8      | 7.3   |
|                      | In situ          | 5      | 4.6   |

Every fresh specimen was stained for 10 s with a fluorochromatic dye which binds to nucleic acids and negatively charged proteins (Histolog Dip®), and subsequently rinsed in a phosphate buffer solution. The specimen was then placed on a special transparent dish (Histolog® Dish) and applied on the scanning system for laser analysis. Except for a very large specimen (10 × 6 cm), the imaging area (46 × 36 mm) allowed full viewing of the specimens in a single window. As Histolog® Scanner V2 only detects a single plane, it was essential to bring the area to scan exactly into contact with the surface of the dish. Therefore, in addition to tissue dissection (when necessary), further flattening was achieved by exerting pressure on the specimen (using a flour-filled vinyl glove, e.g.). Fast (15 s), low-quality “preview” imaging was used for immediate evaluation of optimal specimen positioning and flattening. Once obtained, a longer (1 min), high-quality image was taken for subsequent analysis. Regularly, surgical specimens had to be re-positioned for optimal viewing of different parts of the specimen in case of irregular flattening for example. Of note, suboptimal resolution was observed in case of insufficient flattening of the specimen or too much pressure exerted on the specimen. The images were then anonymized and saved for later analysis. After image creation, the specimen was fixed in 4% formaldehyde for standard histology processing or frozen and OCT-embedded for per-operative Mohs analysis.

Confocal digital images were analyzed using Histolog® software on Histolog® Scanner V2 (to mimick immediate per-operative assessment), by LG and FK with no prior preparation. For confocal analysis, BCC-specific criteria included demarcated fluorescent areas with higher nuclear density, peripheral palisading, clefting and nuclear polymorphism.14 For SCC, confocal criteria included “black” sharply demarcated irregular areas in the epidermis and dermis (erosion/ulceration), disarray of the normal architecture of the skin, irregular aggregates of cells that are larger than inflammatory cells, keratin pearls and peritumoral inflammatory infiltrate.15,16 To facilitate digital image analysis, LG and FK used the zoom, contrast and black/white versus purple (H&E-like) digital modes allowed by the Histolog® software. Every confocal image was analyzed by two examiners and scored according to quality (very low, low, high, very high quality), based on the following criteria: the displaying of the whole epidermis (for peripheral margins); the absence of “air bubbles”; the absence of blurred areas (due to haemorrhage or to movement-induced stitched mosaic artefacts) or areas of lower resolution (likely caused by suboptimal contact of the specimen with the dish, in case of insufficient flattening or too much pressure exerted on the specimen). Very high, high, low and very low quality were attributed according to the proportion (<5%, 5-10%, 10-30% and >30%
respectively) of the analyzed area harbouring the above-mentioned criteria. The times required to obtain (including staining and flattening process) and analyze the definitive images were registered. Confocal diagnoses were compared to conventional histopathological H&E analyzes. The performance of ex vivo CLSM was evaluated by calculating sensitivity, specificity, positive and negative predictive values, both globally, and according to tumour or surgical specimen types.

3 | RESULTS

The mean time for specimen processing (including flattening) and acquisition of the image was 5.1 ± 3.4 min (range: 2–15 min). We obtained 89% (n = 97) high and very high quality images, with 61% (n = 67) of very high quality images (Table 2). Both BCC and SCC confocal features were recognized (Figures 2–4). The mean time for confocal image analysis was 1 ± 0.76 min (range: 30 s–5 min). The diagnostic sensitivity and specificity of ex vivo CLSM compared to classical H&E procedures were respectively 93% (95% CI: 66.1%–99.8%) and 100% (95% CI: 15.8%–100%) when assessed on tumour biopsies. Consistently with previous reports, we found higher sensitivity for BCC (100%; 95% CI: 66.4%–100%) than for SCC (80%; 95% CI: 28.4%–99.4%; Table 3). Calculated positive and negative predictive values were both 100% for BCC biopsies, while 100% and 50% respectively for SCC biopsies.

The global sensitivity and specificity for surgical margin assessment using ex vivo CLSM compared to classical H&E procedure were respectively 61.5% (95% CI: 31.6%–86.1%) and 95% (95% CI: 87.7%–98.6%; Table 3). Again, we found higher sensitivity (80%; 95% CI: 28.4%–99.4%) and specificity (100%; CI: 90.6%–100%) for BCC than for SCC (50%; 95% CI: 15.7%–84.3%, and 91%; 95% CI: 77.9%–97.4%, respectively; Table 3). Calculated positive and negative predictive values for surgical margins assessment were 100% and 97% for BCC, while 50% and 90.7% for SCC. Regarding the different types of surgical margins, sensitivity and specificity were respectively 50% (95% CI: 1.3%–98.7%) and 95% (95% CI: 81.8%–99.3%) for deep margins (fusiform excision and slow-

TABLE 2  Quality assessment of confocal digital images

| Image quality | Number | % |
|---------------|--------|---|
| Very high     | 67     | 61.5 |
| High          | 30     | 27.5 |
| Low           | 8      | 7.3  |
| Very low      | 4      | 3.7  |

FIGURE 2  Confocal laser scanning microscopy (CLSM) image (a) and corresponding H&E staining (b) of a basal cell carcinoma (BCC). Scale bars indicate 1000 µm in the bigger panels and 200 µm in the smaller panel

FIGURE 3  Confocal laser scanning microscopy (CLSM) image (a) and corresponding H&E staining (b) of a squamous cell carcinoma (SCC). Scale bars indicate 1000 µm in the bigger panels and 200 µm in the smaller panel
Mohs bases compartment), 50% (95% CI: 6.7%–93.2%) and 92% (95% CI: 74.9%–99%) for peripheral margins (slow-Mohs lateral compartments), and 71% (95% CI: 29%–96.3%) and 100% (95% CI: 80.5%–100%) for Mohs excisions (Table 3). Calculated positive and negative predictive values were 33.3% and 97.2% for deep margins, 50% and 92.3% for peripheral margins and 100% and 89.5% for Mohs specimens.

4 | DISCUSSION

Here, we report the sensitivity and specificity for surgical margin assessment of skin cancers using the improved version of the CLSM Histolog® Scanner (V2). Overall, our study reports high quality images in most cases (89%) after adequate flattening and positioning of the tissue. Stitched mosaics previously reported with other microscopes were rarely observed. Globally, we found similar sensitivity and specificity in surgical margin assessment when compared to conventional H&E histopathological analyzes than previous reports performed with different microscopes. In particular, despite the limited penetration depth (approximately 30 μm), we report similar sensitivity (80% vs. 79.8%–99%) and specificity (100% vs. 80%–100%) for BCC detection compared to VivaScope2500, while lower sensitivity (50% vs. 95%) and specificity (91% vs. 96%) for SCC detection. Thus, given its larger field of view, its accessibility for up to 10 cm-size specimens, and its faster acquisition times ("preview" 15 s, "acquisition" 1 min), Histolog® Scanner V2 appears particularly suitable for the surgical margins assessment of large BCC specimens.

When looking specifically into BCC surgical margins, we report excellent positive and negative predictive values (100% and 97% respectively), further establishing the potential role of ex vivo CLSM for BCC surgical margin assessment in the clinics. Importantly, the false negative case was a highly infiltrative (sclerodermiform) BCC composed of very thin tumours islands, which were missed on the confocal digital image (Figure 5,

**Figure 4** Confocal laser scanning microscopy (CLSM) image (a) and corresponding H&E staining (b) of a Mohs surgical specimen. Dotted circles indicate residual BCC foci. Scale bars indicate 1000 μm

**Table 3** Sensitivity and specificity of ex vivo CM using Histolog Scanner compared to histologic analysis on paraffin-embedded or frozen (Mohs) H&E sections

|                      | n | True positive | False positive | True negative | False negative | Sensitivity (%) | Specificity (%) |
|----------------------|---|---------------|----------------|---------------|----------------|----------------|----------------|
| Biopsies             | 16| 13            | 0              | 2             | 1              | 93 (CI: 66.1%–99.8%) | 100 (CI: 15.8%–100%) |
| Tumour type          |    |                |                |               |                |                |                |
| BCC                  | 10| 9             | 0              | 1             | 0              | 100 (CI: 66.4%–100%) | 100 (CI: 2.5%–100%) |
| SCC                  | 6 | 4             | 0              | 1             | 1              | 80 (CI: 28.4%–99.4%) | 100 (CI: 2.5%–100%) |
| Surgical margins     | 93| 8             | 4              | 76            | 5              | 61.5 (CI: 31.6–86.1) | 95 (CI: 87.7–98.6) |
| Tumour type          |    |                |                |               |                |                |                |
| BCC                  | 42| 4             | 0              | 37            | 1              | 80 (CI: 28.4–99.4) | 100 (CI: 90.6–100) |
| SCC (including BSC)  | 51| 4             | 4              | 39            | 4              | 50 (CI: 15.7–84.3) | 91 (CI: 77.9–97.4) |
| Surgical specimen type | |                |                |               |                |                |                |
| Depth                | 39| 1             | 2              | 35            | 1              | 50 (CI: 1.3–98.8) | 95 (CI: 81.8–99.3) |
| Periphery            | 30| 2             | 2              | 24            | 2              | 50 (CI: 6.8–93.2) | 92 (CI: 74.9–99)  |
| Mohs                 | 24| 5             | 0              | 17            | 2              | 71 (CI: 29–96.3) | 100 (CI: 80.5–100) |

Abbreviation: CI, 95% confidence intervals.
upper panels). Previous data report the detection by ex vivo CLSM of very thin infiltrative BCC tumour islands in perineural or perivascular invasion,17 using however other confocal microscopes combining reflectance and fluorescence mode with possible deep scanning into the specimen. This example illustrates the limited resolution offered by the Histolog® Scanner V2, and the difficulty of recognizing highly infiltrative BCC foci. Although encouraging, the analysis of SCC specimens highlighted the lower positive and negative predictive values (50.0% and 90.7% respectively) for margin assessment using ex vivo CLSM, reflecting its more difficult histopathological analysis.

Our study also reports the specific diagnostic accuracy of ex vivo CSLM for biopsies, deep and peripheral margins or Mohs specimens. While we found high diagnostic sensitivity (93%; 95% CI: 66.8%–99.3%) and specificity (100%; 95% CI: 15.8%–100%) for biopsies, the negative predictive value was relatively low (66.7%), mostly due to the low number of tumour-free and the difficult positioning/flattening of thin, firm biopsy samples, hence lower image quality. Deep and peripheral margins assessment revealed low diagnostic accuracy with 33.3% and 50% positive predictive value respectively, but higher negative predictive values (97.2% and 92.3% respectively). In particular, the one false negative deep margin resulted from a highly infiltrative basosquamous carcinoma (Figure 6). At this point, it is unclear whether this resulted from the limited detection by Histolog® Scanner V2 of very small tumour cell clusters, or from a histological “false positive” as no tumour island was found in the 30 μm deepest margin, analyzed by CLSM. Of note, the “false negative” cases observed in the peripheral margins assessment, may actually represent histological “false positive” from tissue loss during paraffin-embedded tissue sectioning during conventional histopathological procedure, as previously reported.11 In the contrary, the “false positive” observed in the deep margin assessment using ex vivo CLSM may actually represent histological “false negative”, due to the limited (1%–2%) margin assessment of deep margins during conventional H&E examining.3 Remarkably, the assessment of Mohs specimens revealed higher sensitivity (71%) and specificity (100%) compared to deep and peripheral margin assessment, with positive and negative predictive values of 100% and 89.5% respectively. In favour of higher diagnostic accuracy are the debulking and flattening expertise of the technicians for Mohs specimens. As a limitation however, confocal images and corresponding Mohs slides were examined by the same reviewer. Notably, the 2 false negative cases were due to a highly infiltrative (sclerodermiform) BCC sample (previously discussed; Figure 5, upper panels) and a residual in situ SCC on the peripheral border (Figure 5, lower panels).

Having these limitations in mind, our data suggest that ex vivo CLSM is a fast and accurate alternative to analyze surgical margins, although with yet limited resolution (in particular for small cell clusters of highly

![Figure 5](image-url) Confocal laser scanning microscopy (CLSM) images (a,c) and corresponding H&E stainings (b,d) of Mohs surgical specimens. (a) and (b) illustrate a false-negative CLSM picture of a highly infiltrating BCC. Arrowheads indicate tumour cell clusters on H&E (b) that were missed on the CLSM picture (a). (c) and (d) illustrate a false-negative CLSM picture of a residual peripheral in situ SCC. Dotted lines indicate the magnified areas. Scale bars indicate 1000 μm in the bigger panels and 200 μm in the smaller panels.
infiltrative tumours). Currently, our data suggest that ex vivo CLSM using Histolog® Scanner V2 is mostly suited for the assessment of non-infiltrative BCCs margins, especially when conventional histopathological analysis (prone to false negative because of limited margin assessment) is used. Further prospective studies will be needed to improve and refine the place of ex vivo CLSM in the future.

ACKNOWLEDGEMENTS
We thank SamanTree Medical SA, Lausanne, Switzerland who provided the microscope (Histolog® Scanner V2), the Histolog® Dishes and Histolog® Dip. We thank Isabelle Surbeck and Ana Joncic for processing the surgical specimens before image acquisition on the CLSM. We thank Laura Yerly for the digital scanning of the H&E stainings.

CONFLICT OF INTEREST
None to declare.

AUTHOR CONTRIBUTIONS
Lorenzo Grizzetti: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing; Francois Kuonen: Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

DATA AVAILABILITY STATEMENT
All data will be available upon request.

ETHICS STATEMENT
The study design was approved by the institutional review board of Lausanne University Hospital CHUV, and the local ethics committee (study 2015-00187). Each patient enrolled provided written informed consent.

ORCID
F. Kuonen https://orcid.org/0000-0001-6137-7483

REFERENCES
1. Kim JYS, Kozlow JH, Mittal B, Moyer J, Olencki T, Rodgers P, et al. Guidelines of care for the management of basal cell carcinoma. J Am Acad Dermatol. 2018;78(3):540–59.
2. Kim JYS, Kozlow JH, Mittal B, Moyer J, Olencki T, Rodgers P, et al. Guidelines of care for the management of cutaneous squamous cell carcinoma. J Am Acad Dermatol. 2018;78(3):560–78.
3. Tolkachjov SN, Brodland DG, Coldiron BM, Fazio MJ, Hruza GJ, Roenigk RK, et al. Understanding Mohs micrographic surgery. Mayo Clin Proc. 2017;92(8):1261–71.
4. Malvehy J, Pérez-Anker J, Toll A, Pigem R, Garcia A, Alos LL, et al. Ex vivo confocal microscopy: revolution in fast pathology in dermatology. Br J Dermatol. 2020;183(6):1011–25.
5. 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.
6. Larson B, Abeytunge S, Seltzer E, Rajadhyaksha M, Nehal K. Detection of skin cancer margins in Mohs excisions with high-speed strip mosaicing confocal microscopy: a feasibility study. Br J Dermatol. 2013;169(4):922–6.
7. Bennassar A, Villata A, Puig S, Malvehy J. Ex vivo fluorescence confocal microscopy for fast evaluation of tumour margins during Mohs surgery. Br J Dermatol. 2014;170(2):360–5.
8. Mu EW, Lewin JM, Stevenson ML, Meehan SA, Carucci JA, Gareau DS. Use of digitally stained multimodal confocal mosaic images to screen for nonmelanoma skin cancer. JAMA Dermatol. 2016;152(12):1335.
9. Espinasse M, Cinotti E, Grivet D, Labeille B, Prade V, Douchet C, et al. ‘On face’ ex vivo reflectance confocal microscopy to help the surgery of basal cell carcinoma of the eyelid: confocal microscopy for eyelid tumours. Clin Experiment Ophthalmol. 2017;45(5):442–7.
10. Longo C, Pampena R, Bombonato C, Gardini S, Piana S, Mirra M, et al. Diagnostic accuracy of ex vivo fluorescence confocal microscopy in Mohs surgery of basal cell carcinomas: a prospective study on 793 margins. Br J Dermatol. 2019;180(6):1473–80.
11. Peters N, Schubert M, Metzler G, Geppert J-P, Moehrle M. Diagnostic accuracy of a new ex vivo confocal laser scanning microscope compared to H&E-stained paraffin slides for micrographic surgery of basal cell carcinoma. J Eur Acad Dermatol Venereol. 2019;33(2):298–304.
12. Horn M, Gerger A, Koller S, Wegener W, Langsenlehner U, Kripp P, et al. The use of confocal laser-scanning microscopy in microsurgery for invasive squamous cell carcinoma. Br J Dermatol. 2007;156(1):81–4.
13. Moehrle M, Breuninger H, Röcken M. A confusing world: what to call histology of three-dimensional tumour margins? J Eur Acad Dermatol Venereol. 2007;21(5):591–5.
14. Ziefe S, Schüle D, Breuninger H, Schippert W, Moehrle M. Confocal laser scanning microscopy vs 3-dimensional histologic imaging in basal cell carcinoma. Arch Dermatol. [Internet]. 2010;146(8):843–7. https://doi.org/10.1001/archdermatol.2010.191
15. Hartmann D, Krammer S, Bachmann MR, Mathemeier L, Ruzicka T, Bagci IS, et al. Ex vivo confocal microscopy features of cutaneous squamous cell carcinoma. J Biophotonics. 2018;11(4):e201700318.
16. Longo C, Ragazzi M, Gardini S, Piana S, Moscarella E, Lallas A, et al. Ex vivo fluorescence confocal microscopy in conjunction with Mohs micrographic surgery for cutaneous squamous cell carcinoma. J Am Acad Dermatol. 2015;73(2):321–2.
17. Pérez-Anker J, Ribero S, Yélamos O, García-Herrera A, Alos L, Alejo B, et al. Basal cell carcinoma characterization using fusion ex vivo confocal microscopy: a promising change in conventional skin histopathology. Br J Dermatol. 2020;182(2):468–76.

How to cite this article: Grizzetti L, Kuonen F. Ex vivo confocal microscopy for surgical margin assessment: a histology-compared study on 109 specimens. Skin Health Dis. 2022;2(2):e91. https://doi.org/10.1002/ski2.91