In Vivo Real-Time Confocal Reflectance Microscopy: A Noninvasive Guide for Mohs Micrographic Surgery Facilitated by Aluminum Chloride, an Excellent Contrast Enhancer

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BACKGROUND. **Mohs micrographic surgery (MMS) is based on microscopically controlled excision of cutaneous neoplasms and offers the highest cure rates with maximum tissue preservation. In vivo confocal microscopy (CM) allows noninvasive optical imaging of thin sections of living skin, in its native state, in real time, with high resolution and contrast.**

OBJECTIVE. **To evaluate the feasibility of the use of in vivo CM as a surgical guide in MMS.**

METHODS. **Five patients with a biopsy-proven basal cell carcinoma (BCC) were imaged by in vivo CM on one or two sites from the clinically visible skin cancer. The first Mohs layer was then excised, and the fresh-frozen sections were correlated with the CM findings. Aluminum chloride (AlCl) 20% was applied on the Mohs defect followed by in vivo CM on one site from each lesion. A second Mohs layer was subsequently excised, and fresh-frozen sections were correlated with CM findings.**

RESULTS. **The findings of in vivo CM were confirmed by hematoxylin and eosin–stained frozen sections after excisions of the first and second Mohs layers. AlCl was found to provide an excellent contrast between BCC cells and the surrounding tissue, detected readily with both in vivo and ex vivo CM. The tumor cells with AlCl exhibited intensely bright nuclei with an excellent contrast as compared with the low-contrast dark nuclei without AlCl application.**

CONCLUSION. **In vivo CM is a potential surgical guide for MMS, and AlCl provides an excellent exogenous agent to enhance tumor contrast for CM.**

THE CONFOCAL MICROSCOPE WAS LOANED BY LUCID, INC. THIS STUDY WAS PARTIALLY FUNDED BY A GRANT FROM THE U.S. DEPARTMENT OF ENERGY. A PATENT HAS BEEN FILED ON THE USE OF ALUMINUM CHLORIDE TO ENHANCE CONFOCAL MICROSCOPY VISUALIZATION.

THE USE of a noninvasive, in vivo (real-time) reflectance-mode CM for investigating skin malignancies holds the potential to help physicians improve their diagnostic abilities in various clinical settings.

Confocal microscopy (CM) is an imaging technique that can "optically section" biological objects.[1–3] Thin planes of a biological specimen can be imaged at very high resolution and contrasted without physically dissecting the specimen. In the last 10 years, CM has been increasingly used in biology[2] and applied to imaging skin in vivo.[4–7] In 1995, the ability to image nuclear and cellular-level details in human skin in vivo with a video-rate laser-scanning CM constructed in our laboratories (Wellman Laboratories of Photomedicine) was reported, and a detailed description of the in vivo reflectance-mode CM has been published.[8] Outside of our laboratory, other researchers have used the white-light tandem confocal scanning microscope to similarly image human epidermis and superficial dermis.[4–7] Using a near-infrared laser and water immersion objective lenses (numerical aperture = 0.7 to 1.2), thin sections of human skin can be histologically imaged in vivo.[9] CM provides horizontal "en face" sections of the skin instead of the vertical sections that we are familiar within routine histology. The resolution of the images are high (axial resolution—section thickness—of 3 to 5 μm and lateral resolution of better than 1.0 μm) and are comparable to conventional histology reaching down to the papillary and upper reticular dermis. Therefore, real-time CM provides a noninvasive window into living skin for basic and clinical research. Unlike routine histology and confocal fluorescence microscopy, skin is imaged in its native state either in vivo or ex vivo

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ISSN: 1076-0512/03/$15.00/0 • Dermatol Surg 2003;29:839–846

without the fixing, sectioning, and staining required for conventional histology. CM has been used to visualize different benign skin conditions,[10,11] melanoma,[12–14] and nonmelanoma skin cancer, including basal cell carcinoma (BCC).[15]

Mohs micrographic surgery (MMS) is a surgical procedure that is based on microscopically controlled excision of cutaneous neoplasms. It offers the highest cure rates[16–18] among other therapeutic modalities while maximally preserving surrounding normal skin. This modality is the treatment of choice for neoplasms in high-risk locations where functional and cosmetic reconstruction is limited and for histologic tumor subtypes with aggressive biologic behavior. MMS involves excision of the clinically apparent neoplastic lesion, processing, and staining of horizontal frozen sections using reagents such as hematoxylin and eosin or toluidine blue, stepwise microscopic analysis, meticulous mapping of tumor extensions, and re-excision of residual neoplasm until tumor-free margins are obtained. Preparation of frozen stained sections can require approximately 20 to 60 minutes per stage. There are many cases, however, with Mohs' surgery where the tumor extends well beyond the clinical margins.

Combining reflectance CM with MMS might help improve the accuracy of MMS, minimize its invasiveness, and result in less sacrifice of uninvolved tissue. This noninvasive imaging tool can potentially be used for the preoperative diagnosis of nonmelanoma skin cancer[15] and for defining margins before excision by Mohs technique. During Mohs surgery, reflectance CM may also help to demarcate tumor margins for a faster excision and more predictable prognosis after surgical treatment. In addition, the ability to demarcate tumor margins in vivo should lead to less tissue sampling during surgery, thereby minimizing the amount of normal tissue removed and resulting in minimally invasive therapy.

This study was designed to evaluate the feasibility of the use of in vivo reflectance-mode CM as a surgical guide in MMS. In our study, we optimized CM for in vivo imaging during Mohs surgical procedure and correlated in vivo tumor mapping by CM with the invasive histologic map obtained during MMS.

Methods

Study Design

Seven patients were enrolled in this study, two females and five males, with their age ranging between 46 and 80 years. The patient population was recruited after signing an informed consent obtained under an institutional review board–approved protocol. All of the seven patients presented with a biopsy-proven BCC on the face (one on the chin, three on the forehead, one on the cheek, and two on the temple). Histopathologic interpretation of the seven biopsies revealed features that are diagnostic of nodular BCC in three lesions, superficial and nodular BCC in one lesion, and infiltrative BCC in one lesion. Two BCC biopsies were not typed because of the nature of the biopsy specimens (Table 1).

Table 1. Clinical and Histologic Data

Patient Number	*Age/Gender*	*Site*	*Diagnosis*
1	67/M	Left forehead/eyebrow	BCC, ns
2	78/F	Mid forehead	BCC, SN
3	80/M	Left chin	BCC, ns
4	70/M	Left cheek	BCC, N
5	74/F	Left forehead	BCC, N
6	65/M	Left temple	BCC, N
7	46/M	Right temple	BCC, I

ns = not specified; I = infiltrative type; N = nodular type; SN = superficial and nodular types.

We used the commercial version of the reflectance confocal microscope approved by the Food and Drug Administration for in vivo imaging of human skin (Vivascope 1000; Lucid Inc., Henrietta, NY). It uses an 830-nm diode laser and a ×30, 1.9 numerical aperture water-immersion objective lens. The clinically visible margins of each lesion were delineated by a Sharpee marking pen. One to two points were also marked at a midpoint of a peripheral margin and/or the center of the lesion. Refractive index mismatch between the objective lens, immersion media, and the tissue to be visualized causes spherical aberration, which in turns causes a loss of resolution and contrast. In intact normal skin, it has been found that we obtain the best resolution and contrast when the immersion medium has a refractive index that matches the measured value of 1.34 for epidermis.[9] Sterile water has been demonstrated to be the ideal immersion medium on intact skin.[9] Sterile water was applied on the intact lesion followed by the placement of a metal tissue ring to stabilize the tissue and to allow placement of the confocal microscope objective. The center of the ring coincided with one of the marked points (Figure 1). The size of the aperture was approximately 100 μm. Confocal imaging was then performed on the one to two marked points on each lesion (Table 2). A skin nick was created on each marked midpoint of a peripheral margin that had been already imaged by CM. The first Mohs layer was then excised following the visually delineated margins as a guide. The purpose of the skin nick was to act as the corresponding correlate to the imaged point during interpretation of the fresh-frozen sections and to provide a reference

point for accurate placement of the CM objective should a second stage of Mohs surgery be needed because of residual tumor after the first stage. A frozen section was prepared for correlation with the confocal findings to see whether the presence or absence of tumor found with CM could be confirmed histologically. CM provides imaging of the superficial plane of the tissue, up to 230 μm from the level of stratum corneum at a wave length of 830 nm of diode laser. However, the Mohs sections are processed in a way that the deep plane of the tissue is examined by frozen sections. If the deep margin visualized by Mohs technique is free of BCC, the block is removed from the cryostat, melted, flipped upside down, and reprocessed so that new recuts are obtained from the superficial plane of the block. The new superficial recuts are then examined microscopically, and the findings are correlated with CM findings. On the other hand, if the deep plane of the block examined by Mohs technique is involved with BCC, the superficial plane of the block is predicted to be involved with tumor, because of the fact that BCC usually grows in a contiguous manner.

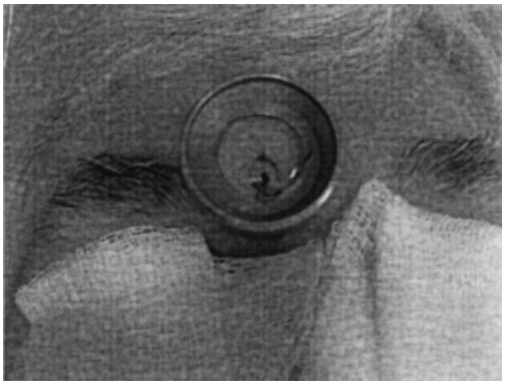


Figure 1. Patient 3, superficial and nodular BCC. The margins and center of BCC are delineated by a marking pen. The tissue ring is applied in preparation to CM.

To control bleeding after excision of the first layer and to minimize refractive index tissue mismatch, aluminum chloride (AlCl) in a 20% solution (Drysol) was applied on the Mohs defect. AlCl is a desiccating agent that is used primarily for the treatment of hyperhidrosis and as a hemostatic agent for superficial wounds. It usually exists in 20% to 40% concentrations mixed with water or alcohol. The most common ready mixed product is a solution of AlCl (hexahydrate) 20% wt/vol in anhydrous ethyl alcohol (Drysol). After AlCl administration and establishment of hemostasis, the wound was rinsed with sterile water, and a layer of sterile water was left on the wound and subsequently covered with Tegaderm dressing. The confocal microscope metal stabilizing ring was then applied on top of the Tegaderm, and sterile water was used to fill the reservoir created by the tissue ring interface.

In vivo CM imaging was then performed on one marked site from each lesion (midpoint of a peripheral margin or the center of the lesion). The second Mohs layer was then excised with a skin nick created at the imaged midpoint of a peripheral margin for better orientation. Also, a frozen section was prepared from the second Mohs layer and was used to confirm the confocal findings.

The refractive index of the skin after removal of the first skin layer during MMS might differ from intact normal skin. To investigate this hypothesis, Mohs surgical defects were previously created using the skin of a hairless albino guinea pig animal model because of its similarity in skin histology with human skin. Different sterile solutions and gels yielding several refractive indices were analyzed. The immersion media analyzed included sterile water, glycerin, surgilube, and petrolatum ointment. Each immersion medium

Table 2.

	CM pre-excision of 1st layer			*Stage I, BCC (+/−)*	*AlCl*	*CM post-excision of 1st layer*			*Stage II, BCC (+/−)*	*Stages/ sections*
Pat #	*CM*	*Site*	*BCC*			*CM*	*Site*	*BCC*		
1	No	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	Yes	Center	+	+	+	Yes	Center	+	+	II(2)
		Mid superior	−	−						
3	Yes	Center	+	+	+	Yes	Center	+	+	III(3)
		Mid superior	+	+						
4	Yes	Center	−	−	+	Yes	Mid superior	−	?(no flip)	II(2)
		Mid lateral	−	−						
5	No	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	Yes	Mid inferior	+	+	+	Yes	Mid inferior	+	+	II(2)
7	Yes	Mid lateral	+	+	+	Yes	Between mid lateral & center	+	+	II(4)

was applied on the dermis after removal of the first skin layer, and clear Tegaderm was placed on top to provide a dry field for the confocal imaging. Subsequently, a layer of saline was applied between the Tegaderm and the objective lens. We found that clear Tegaderm coupled with sterile water or glycerin resulted in a refractive index closely matching that of sterile water applied to intact skin. Sterile water or glycerin provided good-quality confocal images of dermal structures with excellent resolution and contrast, demonstrating refractive indices that match with the refractive index of the dermis. The use of Tegaderm was necessary to avoid cross-contamination of the wound.

Results

Correlation Between In Vivo CM Images and Hematoxylin and Eosin–Stained Frozen Sections

Seven patients were enrolled in the study; however, only five patients could be imaged in vivo by CM because of technical difficulties that were encountered. Confocal imaging was not performed on patients 1 and 5.

In patient 1, the BCC lesion was very close to the eyebrow, with eyebrow hairs interfering with the imaging (adhesive). Patient 5 could not tolerate lying still for the procedure because of her arthritis and chronic back pain. She also complained of discomfort and pressure sensation from the weight of the confocal microscope on her forehead.

Only five of seven patients were imaged with CM, each imaged before excision of the first Mohs layer at one to two sites (total of eight sites). The findings of CM were confirmed by frozen section histology after excision of the first Mohs layer in eight of eight sites (100%; Table 2). AlCl was subsequently applied on the Mohs defects of these five patients, and confocal imaging was performed at five sites, one site per patient. Confocal findings were confirmed by frozen section histology after excision of the second Mohs layer in four of five patients (patients 2, 3, 6, and 7). In one patient (patient 4), confocal imaging did not detect tumor in the superficial plane of the imaged point (midsuperior margin), that is, the plane usually visualized by CM (see Methods section).

In this patient, the deep plane of the mid superior margin of the second Mohs layer, that is, the plane usually examined by frozen sections in MMS, was free of BCC. However, the specimen tissue block was not flipped upside down, preventing us from documenting the absence of the tumor in the superficial plane of the mid superior margin, that is, the plane imaged by CM. Thus, the confocal findings could not be confirmed in the second Mohs layer of patient 4 and may not necessarily have been inaccurate.

Confocal Imaging With AlCl Acting as an Excellent Contrast Enhancer

Confocal imaging performed on the intact lesions in the five patients (patients 2, 3, 4, 6, and 7) before excision of first Mohs layer revealed confocal features of BCC in five of eight examined sites (Table 2). These confocal features consist of islands of tumor cells with characteristic elongated nuclei exhibiting polarization along the same principal axis and high nuclear to cytoplasmic ratios with the oval nuclei appearing dark in color (low contrast) and the scant cytoplasm appearing bright[15] (Figure 2). There was also associated increased vascularity, prominent predominantly mononuclear inflammatory cell infiltrate, and rolling of leukocytes along the endothelial lining of dermal blood vessels.[15,19] These confocal findings were confirmed by fresh-frozen histology after excision of the first Mohs layer in five of five sites, showing the characteristic hematoxylin and eosin features of BCC (Table 2 and Figure 3). Confocal features of BCC were not detected in three of eight examined sites. The absence of BCC at all of these three sites was confirmed by fresh-frozen histology after excision of the first Mohs layer (Table 2).

After excision of the first layer, AlCl was applied on the Mohs defects in vivo in an attempt to stop the bleeding and to provide a dry field for confocal imaging. The AlCl was found to provide an excellent contrast between tumor cells and the surrounding tissue, facilitating detection more readily with CM.

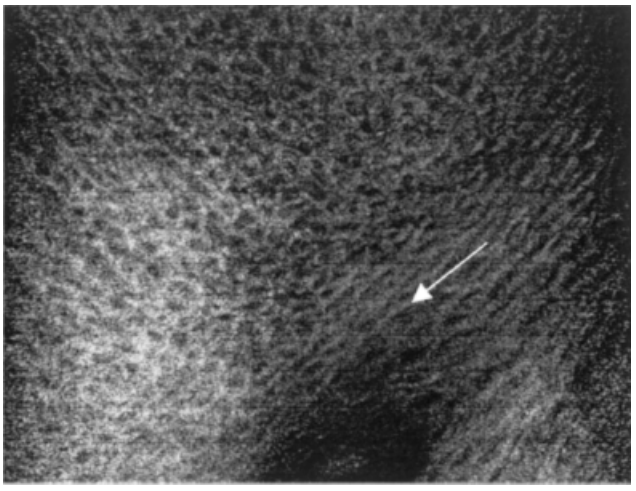
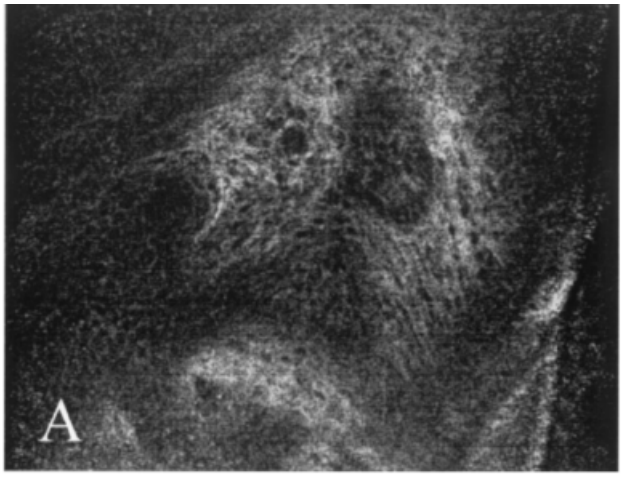
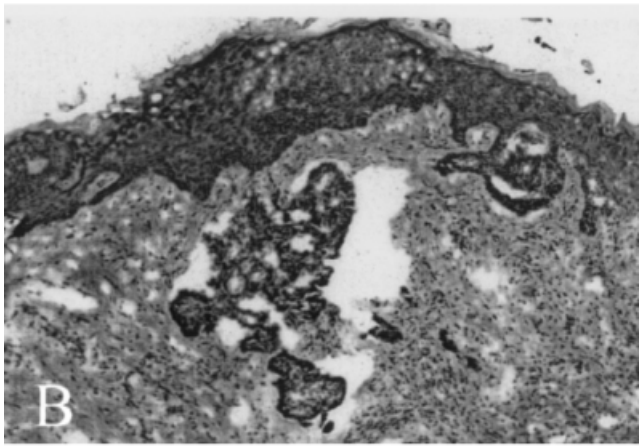


Figure 2. Patient 8, infiltrative BCC, CM pre-excision of first layer. Note the BCC cells with elongated nuclei exhibiting polarization (arrow). Note also the high nuclear to cytoplasmic ratios with the oval nuclei appearing dark in color (low contrast) and the scant cytoplasm appearing bright.





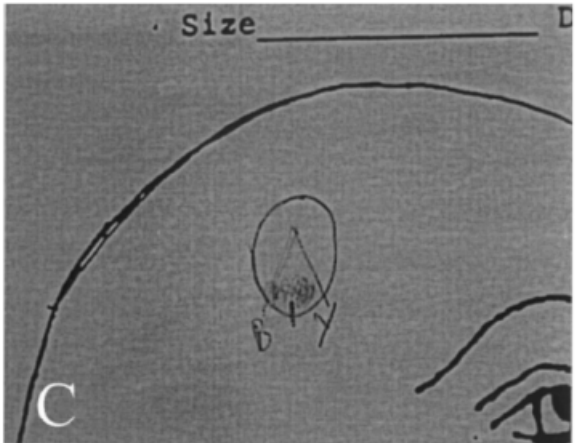


Figure 3. Patient 7, midinferior margin. (A) CM pre-excision of first Mohs layer. Note the characteristic elongated nuclei of BCC (×30, 0.9 numerical aperture). (B) First Mohs layer. Hematoxylin and eosin–stained section confirming the presence of BCC (×20). (C) Marking of the BCC on the invasive histologic map.

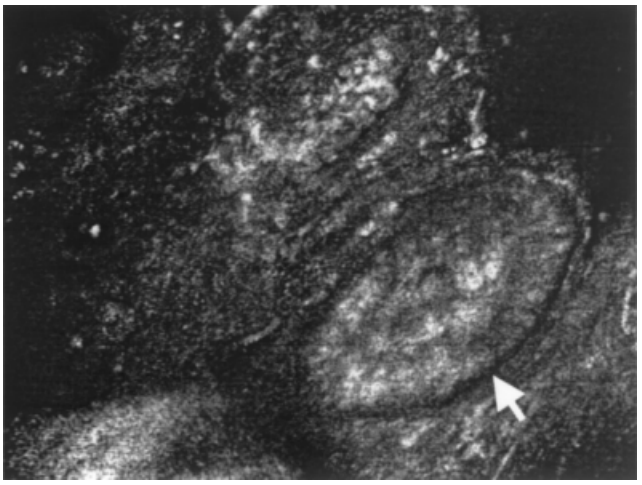
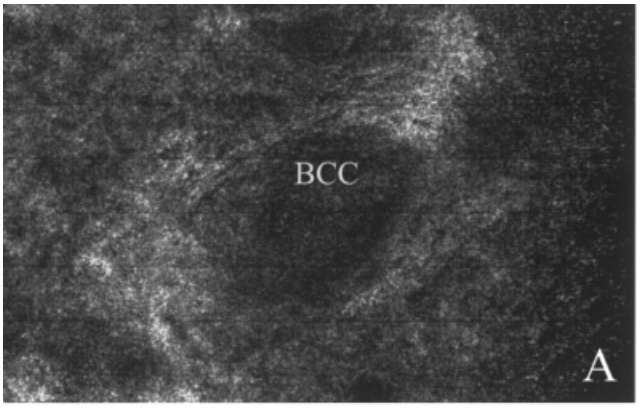


Figure 4. Staining of BCC with AlCl in vivo. Note the excellent contrast between tumor cells and the surrounding tissue, facilitating BCC detection with CM. Note also the clefting between tumor cells and surrounding stroma (arrow, ×30, 0.9 numerical aperture).



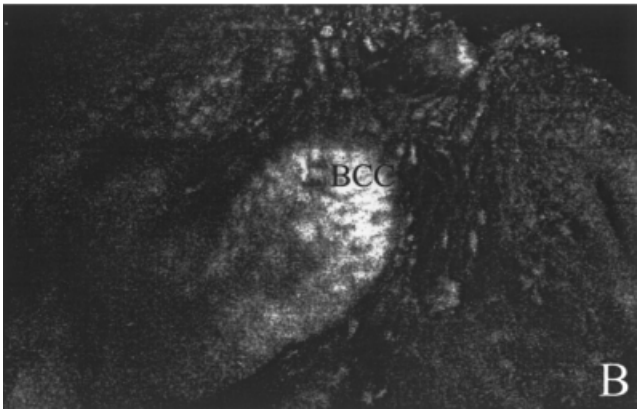


Figure 5. Patient 7, nodular BCC. (A) CM of BCC without AlCl application. (B) CM of BCC after AlCl application. The BCC cells appear intensely bright in contrast to their native dark color and low contrast without AlCl application.

The BCC tumor nodules were more readily defined, with the tumor cells appearing intensely bright in contrast to their native dark color and low contrast without AlCl application (Figures 4 and 5). To elucidate further the effect of AlCl, the clinically visible margins of the infiltrative BCC were marked, and the lesion was divided into two pieces with a surgical pen in patient 7. The midpoint of the lateral

margin of piece 1 was imaged by CM pre-excision and postexcision of the first Mohs layer, and the confocal findings were confirmed by fresh-frozen histology in a fashion similar to the previous four patients (patients 2, 3, 4, and 6; Table 2). A shave biopsy was obtained from the lower half of piece 2, and AlCl was applied on the shaved area, followed by confocal imaging. In vivo CM at that site revealed infiltrative BCC with intensely bright staining of the BCC nuclei. The shaved biopsy specimen was then imaged ex vivo with CM twice: in its native state and after dipping it in AlCl solution. Ex vivo confocal imaging demonstrated the presence of BCC with similar confocal features to those detected by the in vivo confocal imaging. The tumor cells without AlCl appeared to have low-contrast dark nuclei versus intensely bright nuclei with excellent contrast after dipping the specimen in AlCl solution. The latter highlights the usefulness of AlCl as an excellent in vivo and ex vivo contrast enhancer of BCC imaged with CM. Piece 2 was then excised, and fresh-frozen hematoxylin and eosin–stained sections confirmed the presence of infiltrative BCC at the shave biopsy site.

Another interesting finding was the differential uptake of AlCl by tumor cells versus the overlying epidermis. Ex vivo confocal imaging of the shave biopsy (oblique section) after dipping it in AlCl revealed remarkable uptake of AlCl by BCC but no AlCl uptake by the overlying epidermis and stratum corneum (Figure 6A). When compared with another oblique confocal image taken from a peripheral edge of Mohs defect after AlCl application (patient 3), the BCC cells again demonstrated significant uptake of AlCl. There was also some uptake of AlCl by the overlying epidermal cells, in contrast to the shave biopsy specimen where epidermal cells showed no uptake of AlCl (Figure 6B).

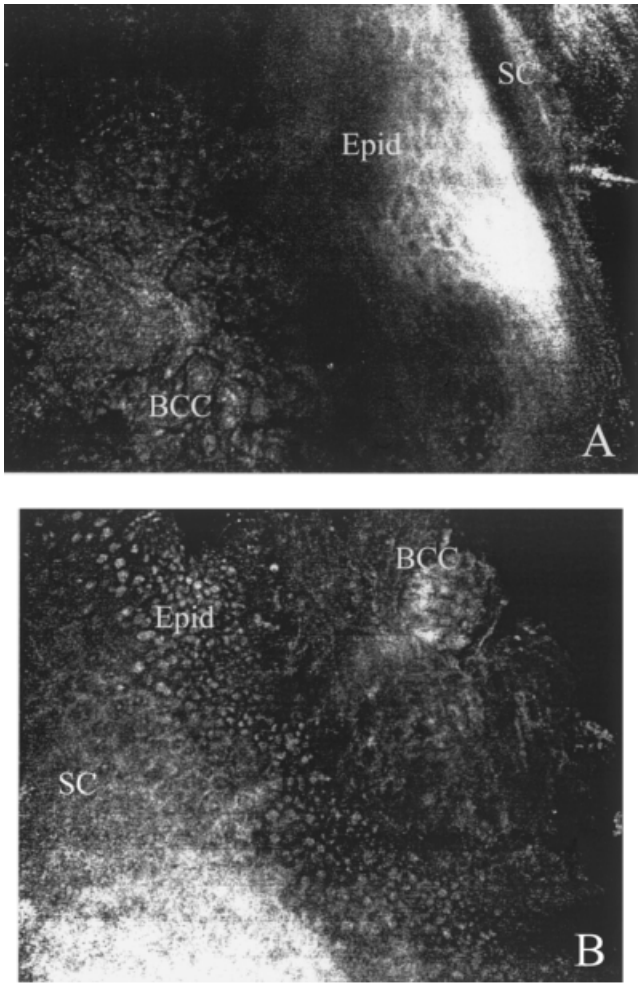


Figure 6. The differential uptake of AlCl by tumor cells versus the overlying epidermis. (A) Ex vivo CM of the shave biopsy after dipping it in AlCl. Note the significant uptake of AlCl by BCC but not by the overlying epidermis and stratum corneum (SC). (B) In vivo CM of a peripheral edge of Mohs defect after AlCl application. Note again the marked uptake of AlCl by the BCC cells. In addition, the overlying epidermal cells also demonstrated some uptake of AlCl.

Discussion

In this pilot study, we have demonstrated that in vivo reflectance-mode CM is a potential guide for MMS. We were able to optimize confocal microscopy for in vivo imaging after excision of the first Mohs layer by using sterile water and Tegaderm applied directly on the Mohs defect. Sterile water and glycerin proved to be the best immersion media with matching refractive indices to that of the dermis among the different immersion media that we have analyzed (guinea pig experiment). We elected to use sterile water in our study instead of glycerin because of its low cost and common sterile availability. Tegaderm applied on the Mohs defect provided a sterile field and prevented cross-contamination of the wound or the microscope without impairing the quality of confocal images.

In vivo CM mapping correlated very well with the invasive histologic map with 100% sensitivity in stage I of MMS (eight of eight sites) and 80% sensitivity in stage II (four of five sites). However, if patient 4 is excluded, the sensitivity would have been 100% for stage II because the tissue block for patient 4 may not have been adequately processed for the study purposes. Thus, confocal reflectance microscopy for in vivo imaging during Mohs surgery holds the possibility to be a noninvasive guide for MMS. Our confocal images correlated very well with the corresponding fresh-frozen hematoxylin and eosin–stained sections, whether on intact lesions or on the Mohs defect after excision of the first Mohs layer. However, only a very

limited area of the total tumor margin was evaluated (midpoint of a peripheral margin or the center of the lesion) because the process is cumbersome and time consuming with the commercial confocal system used in this study (Vivascope 1000). However, a new built-in scanner as well as the new handheld prototype that is currently under development might make this process much easier and less time consuming. If the latter comes to pass, in vivo reflectance CM could improve MMS or other surgery by resulting in less sacrifice of normal tissue through in vivo localization of tumor. As for optimizing CM during in vivo imaging, we have discovered that AlCl 20% (Drysol) applied on a post-Mohs surgery wound or freshly excised skin provides an excellent exogenous agent to enhance tumor contrast for both in vivo and ex vivo CM improving the detection of any remaining tumor that needs to be removed. The tumor cells appear to demonstrate greater uptake of AlCl as compared with normal surrounding skin, with their nuclei appearing intensely bright. This is probably because of back scattering from AlCl-stained cells. Thus, it may be useful not only with CM but also with a CCD camera that would allow screening of larger fields. However, when imaging skin with intact epidermis and stratum corneum before a biopsy or excision is performed, AlCl penetration might be hindered by stratum corneum. We base this on our finding that a freshly excised tissue dipped into AlCl solution with intact stratum corneum did not show any uptake of AlCl by the epidermis. This might be a limitation to AlCl use in in vivo CM on intact skin. However, we hypothesize that this limitation might be easily overcome by removing the stratum corneum (e.g., tape stripping) before application of AlCl. Further experiments are needed to evaluate this hypothesis.

The use of AlCl may also hold promise for application in medical fields other than dermatology. An example could be in needle core biopsies of the breast where AlCl can be applied on fresh biopsy to screen for the presence of tumor epithelium, replacing the time-consuming process of freezing and staining with standard hematoxylin and eosin methods. One drawback of the use of AlCl in vivo is that it might delay wound healing by delaying re-epithelialization.[20] However, that study was based on using 30% AlCl concentration. Future studies with variable concentrations of AlCl have to be done to test whether lower concentrations of AlCl will not affect wound healing significantly while still providing good contrast for the tumor nuclei.

Another contrast agent, 5% acetic acid, was recently used on excised Mohs tissue layers to enhance the contrast of nuclei relative to the surrounding cytoplasm and dermis.[21] The BCC nuclei appeared intensely bright, similar to their appearance after AlCl application. However, unlike AlCl, which can be applied in vivo on intact skin or on open superficial wounds, acetic acid cannot be used in vivo because it causes chemical burns of the skin structures.

Conclusions

The use of in vivo reflectance CM to guide tissue removal during MMS is feasible but currently not very practical. There are some limitations to the use of the current CM (Vivascope 1000) in MMS. It can be time consuming, especially if the entire margins of the lesion are to be evaluated. Incorporating a scanner will solve this problem by providing a wider field of view of the specimen. Another limitation is the inflexible and large tissue ring, which makes imaging of convex and small surfaces unfeasible. A third limitation may be the heavy weight of the Vivascope head, which might not be tolerated by some patients. The latter may change with the new handheld confocal microscope.

The use of AlCl during CM appears to enhance contrast and promises to be useful for skin surgery as well as for other medical uses as well.

References

1. Wilson T, ed. Confocal Microscopy. San Diego: Academic Press, 1990.
2. Pawley JB, ed. Handbook of Biological Confocal Microscopy, 2nd ed. New York: Plenum Press, 1995.
3. Webb RH. Confocal optical microscopy. Rep Prog Phys 1996;59:427–71.
4. New KC, Petroll WM, Boyde A, et al. In vivo imaging of human teeth and skin using real-time confocal microscopy. Scanning 1991;13:369–72.
5. Corcuff P, Leveque JL. In vivo vision of the human skin with the tandem scanning microscope. Dermatology 1993;186:50–4.
6. Corcuff P, Betrand C, Leveque JL. Morphometry of human epidermis in vivo by real-time confocal microscopy. Arch Dermatol Res 1993;285:475–81.
7. Bertrand C, Corcuff P. In vivo spatio-temporal visualization of the human skin by real-time confocal microscopy. Scanning 1994;16:150–4.
8. Rajadhyaksha M, Grossman M, Webb R, Anderson RR. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. 1995;104:946–52.
9. Rajadhyaksha M, González S, Zavislan J, Anderson RR, Webb RH. In vivo confocal scanning laser microscopy of human skin: II: advances in instrumentation and comparison to histology. J Invest Dermatol 1999;113:293–303.
10. González S, González E, White WM, Rajadhyaksha M, Anderson RR. Allergic contact dermatitis: correlation of in vivo confocal imaging to routine histology. J Am Acad Dermatol 1999;40:708–13.
11. González S, Rajadhaksha M, Rubinstein G, Anderson RR. Characterization of psoriasis in vivo by reflectance confocal microscopy. J Med 1999;30:337–56.
12. Langley RG, Rajadhyaksha M, Dwyer PJ, et al. Confocal scanning laser microscopy of benign and malignant melanocytic skin lesions in vivo. J Am Acad Dermatol 2001;45:365–76.
13. Bussam KJ, Hester K, Charles C, et al. Detection of clinically amelanotic malignant melanoma and assessment of its margins by

in vivo confocal scanning laser microscopy. Arch Dermatol 2001;137:923–9.
14. Tannous Z, Mihm M, Flotte T, González S. In vivo examination of lentigo maligna, in situ malignant melanoma, lentigo maligna type by near-infrared confocal microscopy: comparison of confocal images with histologic sections. J Am Acad Dermatol 2002;46:260–3.
15. González S, Tannous Z. Real-time, in vivo confocal reflectance microscopy of basal cell carcinoma. J Am Acad Dermatol 2002;47:869–74.
16. Mohs FE, eds. Chemosurgery: A Microscopically Controlled Method of Cancer Excision. Springfield, IL: Charles C. Thomas, 1978.
17. Robins P. Chemosurgery: my 15 years of experience. J Dermatol Surg 1981;7:779.
18. Rowe DE, Carroll RJ, Day CL Jr. Mohs surgery is the treatment of choice for recurrent (previously treated) basal cell carcinoma. J Dermatol Surg Oncol 1989;15:424–31.
19. González S, Sackstein R, Anderson RR, Rajadhyaksha M. Real-time evidence of in vivo leukocyte trafficking in human skin by reflectance confocal microscopy. J Invest Dermatol 2001;117: 384–6.
20. Sawchuk WS, Friedman KJ, Manning T, Pinnell SR. Delayed healing in full-thickness wounds treated with aluminum chloride solution: a histologic study with evaporimetry correlation. J Am Acad Dermatol 1986;15(5 Pt 1):982–9.
21. Rajadhyaksha M, Menaker G, Flotte T, Dwyer P, González S. Confocal examination of non-melanoma cancers in skin excisions to potentially guide Mohs micrographic surgery without histopathology. J Invest Dermatol 2001;117:1137–43.