Confocal laser microscopic imaging of actinic keratoses in vivo: A preliminary report

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Background: Real-time near-infrared confocal laser scanning microscopy (CM) offers an unprecedented method for confirming the clinical diagnosis of actinic keratosis (AK) without biopsy.

Methods: Seven patients with clinically diagnosed AK underwent CM imaging over the lesion and over adjacent normal-appearing skin. Biopsy specimens were obtained from the presumed AKs in 4 patients.

Results: CM detected lesional pathologic features of hyperkeratosis (71%), lower epidermal nuclear enlargement and pleomorphism (100%), and architectural disarray (57%). In contrast, cytologic atypia and architectural disarray were apparent in one patient (17%) over the adjacent, clinically normal skin. Three of 4 biopsy specimens confirmed the clinical diagnosis of AK, whereas one revealed invasive squamous cell carcinoma. Without optimizing CM for imaging hyperkeratotic skin lesions, the limited depth of penetration reached the stratum basale in only 3 lesions, precluding detection of dermal invasion in the others.

Conclusion: Depth of penetration currently imposes a major limitation on CM in the diagnosis of AKs, especially in hypertrophic and hyperkeratotic lesions, which are more likely to be malignant. However, CM may become an alternative to biopsy, and its limitations may be overcome by future technologic advances in optical penetration or by simply removing the hyperkeratotic stratum corneum. (J Am Acad Dermatol 2000;43:42-8.)

Actinic keratosis (AK) is the most common precancerous lesion of the skin, becoming more prevalent with increasing age.[1] Of fair-skinned people, 60% experience AKs by age 40,[2] and 80% by age 60.[3] Resulting from excessive ultraviolet exposure, they occur predominantly on exposed sites, with 80% located on the head, neck, hands, or forearms.[3] Sun avoidance and sunscreens not only prevent new lesions,[3] but increase the rate of regression, which is 25% spontaneously.[4] The average affected patient has 7 to 10 lesions.[3,4]

Pathologically, AKs are defined by partial-thickness cytologic atypia of the epidermis, beginning at the basal layer and stopping short of the granular layer. Specific features of the dysplastic cells include nuclear enlargement and pleomorphism, mitotic figures, altered polarity, abnormal maturation, and dyskeratosis.[2,3] Lesions are often acanthotic, but are always keratotic, with parakeratosis alternating with hyperkeratosis above adnexae.[3]

Squamous cell carcinoma (SCC) involves full-thickness epidermal dysplasia, naturally the next step in the progression from its partial-thickness precursor, the AK, the same way that cervical intraepithelial neoplasia leads to cervical carcinoma.[1] Animal models of skin carcinogenesis and immunohistochemical analysis of mutations in the tumor suppressor gene p53 also support the premalignant nature of AKs.[3,5] Moreover, 27% of SCCs that undergo biopsy arise directly within AKs, and another 56% arise in their close proximity.[4] In fact, it can be argued that AKs are actually SCCs, representing an early point on the continuum of malignancy.

The risk of progression of a single AK to full-thickness SCC remains unknown, but it has been estimated by various studies within a wide range between 0.1% and 20%.[4,5] Of course, this risk depends upon various features of the lesion, with increased risk attributed to thicker lesions, mucosal lesions, and lesions in immunocompromised persons.[3] The incidence of SCC has risen dramatically over the past 2 decades, now occurring at the rate of more than 200,000 per

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year in the United States.[4] Moreover, SCC is responsible for thousands of deaths per year.[3] Fortunately, SCCs arising in AKs have a relatively lower metastatic rate of 2% to 6%.[1,5] Most important, SCC can be cured if recognized early. Therefore screening for and diagnosing SCC and AK continue to be extremely important functions of the dermatologist.[5]

AKs are diagnosed easily and accurately by dermatologists 90% of the time.[5] These red, skin-colored, or tan plaques are surmounted by a heaped-up dry scale, with a characteristic sandpapery feeling on palpation. They may be asymptomatic or may elicit symptoms of pruritus, burning, or pinching. Occasionally, they can be difficult to differentiate from other lesions, including warts, seborrheic keratoses, discoid lupus, porokeratosis, superficial basal cell carcinoma, and SCC. In one study, interobserver agreement between dermatologists with respect to single AKs was extremely poor, in contrast to the significant agreement obtained for patients with multiple lesions.[6] Often, the clinician must perform a biopsy of the lesion in question for a definitive diagnosis.

Advances in reflectance confocal scanning laser microscopy (CM) now offer a noninvasive alternative to biopsy. CM can be performed in vivo, with high resolution and in real time, using a low-power, near-infrared laser beam focused tightly on a specific point in the skin, detecting only the light reflected from the focal point through a pinhole-sized spatial filter. This beam can then be scanned horizontally over a two-dimensional grid to obtain a horizontal microscopic section, where the high lateral resolution of 0.5 to 1 μm is defined by the tight focus of the laser beam and the small size of the pinhole filter. By adjusting the focal length of the beam, the microscope can image a series of horizontal planes stacked vertically, with an axial section thickness of 2 to 5 μm. However, because of the limited penetration of near-infrared light in the skin, the imaging depth in normal skin is limited to at most 300 to 400 μm, with visualization of the epidermis and papillary dermis. The high resolution of the instrument allows for histologic quality images with cellular and subcellular detail, whereas the video rate of 10 to 20 Hz provides high temporal resolution, facilitating the visualization of dynamic processes such as blood flow. No staining or photochemical reactions are required for imaging, so CM does not alter the native skin and can be repeated indefinitely.[7-11]

In this study, we aim to characterize AKs using this new technology of in vivo CM.

METHODS

Subjects

Seven patients with clinically diagnosed AKs were enrolled in the study after informed consent. They included 5 men and 2 women from 47 to 74 years of age. The lesions were located on the back of the hand in 3 patients, on the dorsal aspect of the forearm in 2 patients, on the temple in 1 patient, and on the forehead in 1 patient. Pretreatment evaluation included a relevant dermatologic history, physical examination, and photography of the AKs with a 35-mm camera.

CM imaging

Confocal imaging was performed on one lesion in each patient by means of the commercially available near-infrared confocal scanning laser microscope (Vivascope 1000, Lucid Inc, Henrietta, NY). This device uses a diode laser at 830 nm, with a power less than 26 mW. The 30× objective lens of numerical aperture 0.9 was applied to the skin using either water (refractive index 1.33) or gel (refractive index 1.3335) as an immersion medium. The field of view, measuring 250 μm, was scanned repeatedly over a 5-mm area of lesional skin. In addition, confocal imaging of adjacent skin that appeared to be normal was performed in 6 of the 7 patients. Two healthy control subjects also underwent confocal imaging over the back of the hand, dorsal aspect of the forearm, temple, and forehead to provide a basis for comparison to nonphotodamaged skin.

Conventional histology

In 4 patients, a 2-mm punch biopsy of the AK was performed. Biopsy specimens were embedded in paraffin and bisected, with half of the specimen sectioned vertically in the traditional manner. The other half underwent serial transverse sectioning through the epidermis. All sections were stained with hematoxylin and eosin.

RESULTS

The 7 lesions clinically diagnosed as AK were erythematous and between 5 and 8 mm. Most were only slightly hyperkeratotic clinically and were selected as such to maximize the value of the limited depth of penetration of CM. Attempts to image lesions with more significant hyperkeratosis resulted in an inability to fully visualize the epidermis. Of the 7 lesions selected, 4 supported imaging into the stratum spinosum, whereas only 3 were conducive to imaging through the stratum basale.

Fig 1 exemplifies the histopathology of an AK in traditional vertical section, whereas Fig 2 illustrates representative transverse sections of the same AK, at depths in the epidermis that correspond to the lettered lines in Fig 1. The images at the far left in Fig 2 were obtained by means of conventional histologic examination. The stratum corneum (Fig 2, *A*, *left*)

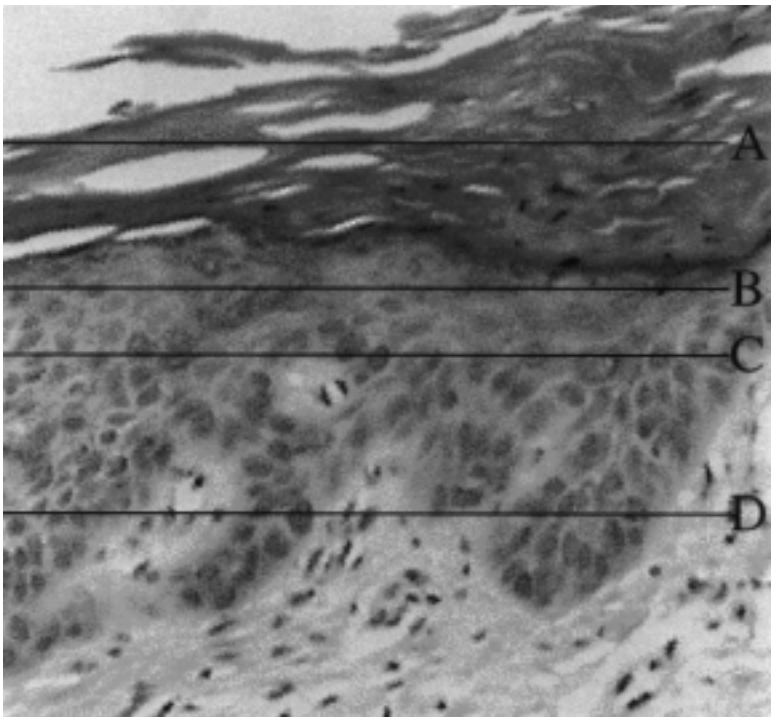

Fig 1. Histopathology of an AK, vertical section. Lines *A, B, C,* and *D* represent depths in the epidermis corresponding to horizontal sections in Fig 2. (Hematoxylin-eosin stain; original magnification ×10; 0.25 numerical aperture, dry objective lens.)

demonstrates thick hyperkeratosis with parakeratosis. Although the stratum granulosum (Fig 2, *B, left*) appears normal, the stratum spinosum (Fig 2, *C*, *left*) and stratum basale (Fig 2, *D*, *left*) show architectural disarray and cytologic atypia with large and pleomorphic nuclei.

The center images in Fig 2 were obtained by CM of the same AK, whereas the images at the far right in Fig 2 were obtained by CM of adjacent normal skin. In the stratum corneum, CM of the AK (Fig 2, *A*, *center*) shows irregular hyperkeratosis, which contrasts with the smooth surface of normal skin (Fig 2, *A*, *right*). Under CM, the stratum granulosum of the AK (Fig 2, *B*, *center*) is composed of uniform, regularly spaced, broad keratinocytes almost identical to those of normal skin (Fig 2, *B*, *right*). Nuclei appear dark in CM images, contrasting with the bright refractile cytoplasm. Under CM, the stratum spinosum (Fig 2, *C*, *right*) and stratum basale (Fig 2, *D*, *right*) of normal skin contain smaller keratinocytes that remain uniform and regularly spaced, unlike in the AK (Fig 2, *C* and *D*, *center*), where the nuclei vary in shape, size, and orientation.

Of the 7 clinically diagnosed AKs imaged with CM, 71% (5/7) demonstrate hyperkeratosis. The stratum granulosum was normal in all lesions except one (14%), in which there was slight variable enlargement of nuclei and architectural disarray. In the stratum spinosum, 100% (7/7) showed clear evidence of nuclear enlargement and pleomorphism, whereas 57% (4/7) manifested architectural disarray. Because of the limited depth of penetration of CM, and because of the thick stratum corneum overlying the lesions, we were able to image as deep as the stratum basale in only 3 of the 7 lesions; of these, all showed evidence of nuclear enlargement and pleomorphism, and 2 of the 3 manifested architectural disarray and elongated dermal papillae.

For comparison, CM of adjacent normal-appearing skin was performed in 6 patients, and hyperkeratosis was found in 50% (3/6). The stratum granulosum was normal in all nonlesional skin. The stratum spinosum and stratum basale showed evidence of nuclear enlargement, pleomorphism, and architectural disarray in only one patient (17%), whose skin was diffusely photodamaged, like many of the other patients in this study. This seemingly normal, but chronically photodamaged skin may, in fact, represent an early AK. None of the nonlesional images demonstrated elongated dermal papillae.

Biopsy specimens were obtained from the imaged lesion in 4 of the 7 patients. Of these, 3 confirmed the diagnosis of AK. One, however, revealed SCC, which was invasive into the superficial dermis, below

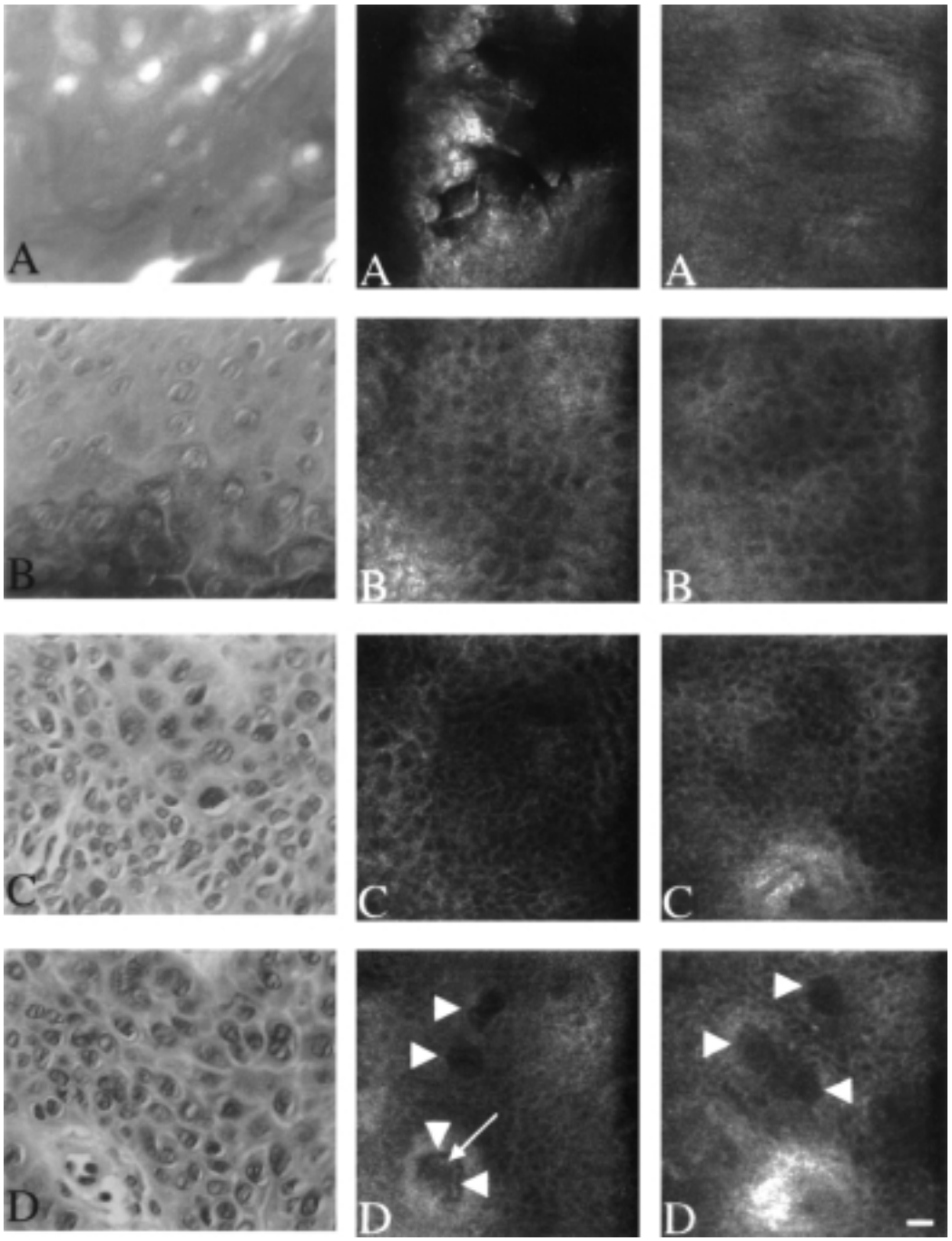

Fig 2. Horizontal sections from depths in epidermis corresponding to lettered lines in Fig 1. *Left column* is conventional histopathology of AK, *center* is CM of AK, and *right column* is CM of adjacent normal skin. Sections were obtained by means of conventional histopathology of AK in Fig 1 (hematoxylin-eosin stain; original magnification ×20; 0.4 numerical aperture, dry objective lens), CM of AK in Fig 1 (×30, 0.9 numerical aperture, water immersion objective lens, scale bar = 25 μm), and CM of adjacent normal skin (original magnification ×30, 0.9 numerical aperture, water immersion objective lens, scale bar = 25 μm). **A,** Stratum corneum. Irregular hyperkeratosis of AK is evident on conventional histopathology and CM, contrasting with smooth surface of normal skin. **B,** Stratum granulosum. Conventional histopathology and CM demonstrate uniform, evenly spaced, broad keratinocytes both in AK and normal skin. In CM images, nuclei appear dark in contrast to bright, refractile cytoplasm. **C,** Stratum spinosum. Conventional histopathology and CM of AK show enlarged, pleomorphic nuclei with haphazard orientation, contrasting with small, uniform, evenly spaced nuclei from normal skin. **D,** Stratum basale. Conventional histopathology and CM of AK show enlarged, pleomorphic nuclei with haphazard orientation, contrasting with small, uniform, evenly spaced nuclei from normal skin. In CM images, dermal papillae appear as well-demarcated, dark holes in epidermis (*arrowheads*), containing blood vessels (*arrow*).

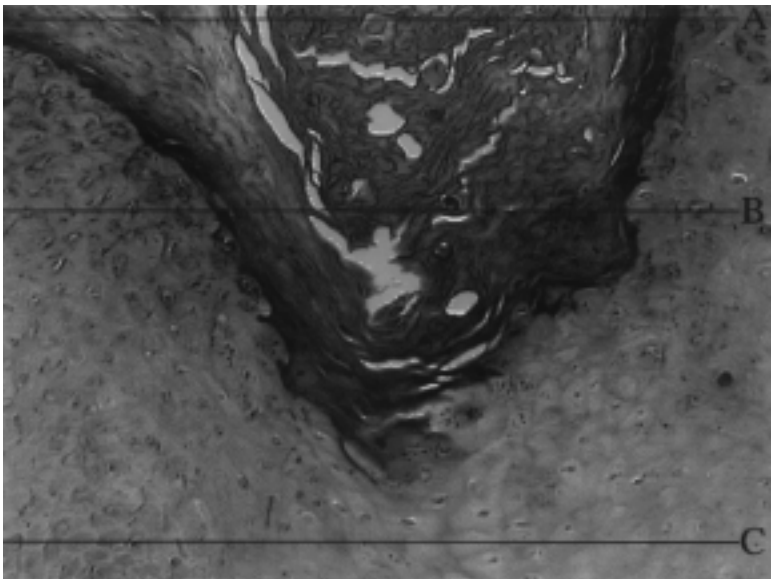

Fig 3. Histopathology of SCC, vertical section. Lines *A, B,* and *C* represent depths in the epidermis corresponding to horizontal sections in Fig 4. (Hematoxylin-eosin stain; original magnification ×4, 0.1 numerical aperture, dry objective lens.)

the imaging depth of CM. Fig 3 shows the superficial histopathology of this lesion in traditional vertical section, whereas the images at the left in Fig 4 are horizontal histopathologic sections of the same lesion. The images at right in Fig 4 were taken during CM of the lesion, at depths corresponding to the lettered lines in Fig 3.

In Figs 3 and 4, the stratum corneum of the SCC (Fig 4, *A*, *left, right*) demonstrates significant hyperkeratosis, accounting for the inability to image deep into the epidermis. Histopathology of the upper epidermis (Fig 4, *B* and *C*, *left*) shows a mass of disorganized pleomorphic keratinocytes with widespread dyskeratosis. The cytologic atypia and architectural disarray are evident in the stratum spinosum on CM (Fig 4, *C*, *right*). However, CM was unable to image deeply enough into the epidermis to visualize the invasion occurring at the dermoepidermal junction. Fortunately, CM was able to characterize a slight variable enlargement of nuclei and architectural disarray in the stratum granulosum (Fig 4, *B*, *right*), distinguishing this lesion from the others clinically diagnosed as AK. Cytologic atypia reaching the top of the epidermis is a defining feature of SCC, and these slight changes on CM in the stratum granulosum (Fig 4, *B*, *right*) attest to the diagnosis of SCC obtained by biopsy in retrospect.

DISCUSSION

The diagnosis and treatment of AK are important because these lesions represent precursors to SCC that can be cured before malignant degeneration. Real-time reflectance CM offers an unprecedented opportunity to differentiate AKs from benign and malignant lesions without biopsy. In this study, CM was able to demonstrate pathologic features of AKs including cytologic atypia and architectural disarray limited to the lower portion of the epidermis. These features were not seen in adjacent normal appearing skin with the exception of one patient with diffuse photodamage, who may have had an early AK in this area.

Unfortunately, depth of penetration imposes a major limitation on CM in the diagnosis of AKs, especially in hypertrophic and hyperkeratotic lesions, which are more likely to be malignant. In practice, the majority of AKs are surmounted by significant hyperkeratosis, which impedes the penetration of CM into the viable epidermal layers. Although, for the purposes of this study, we restricted our imaging to only slightly hyperkeratotic lesions, we were able to image the dermoepidermal junction in only half. CM would offer complete epidermal imaging much less frequently without this artificially imposed restriction. Moreover, confirmation of the clinical diagnosis is less critical for thin, slightly hyperkeratotic AKs because the incidence of SCC rises considerably as lesions become more hypertrophic and hyperkeratotic. For such lesions with more critical diagnostic import, nonoptimized CM performs less well.

The dermoepidermal junction and the stratum basale are important in the pathophysiology of epidermal neoplasms. In AKs, keratinocyte atypia orig-

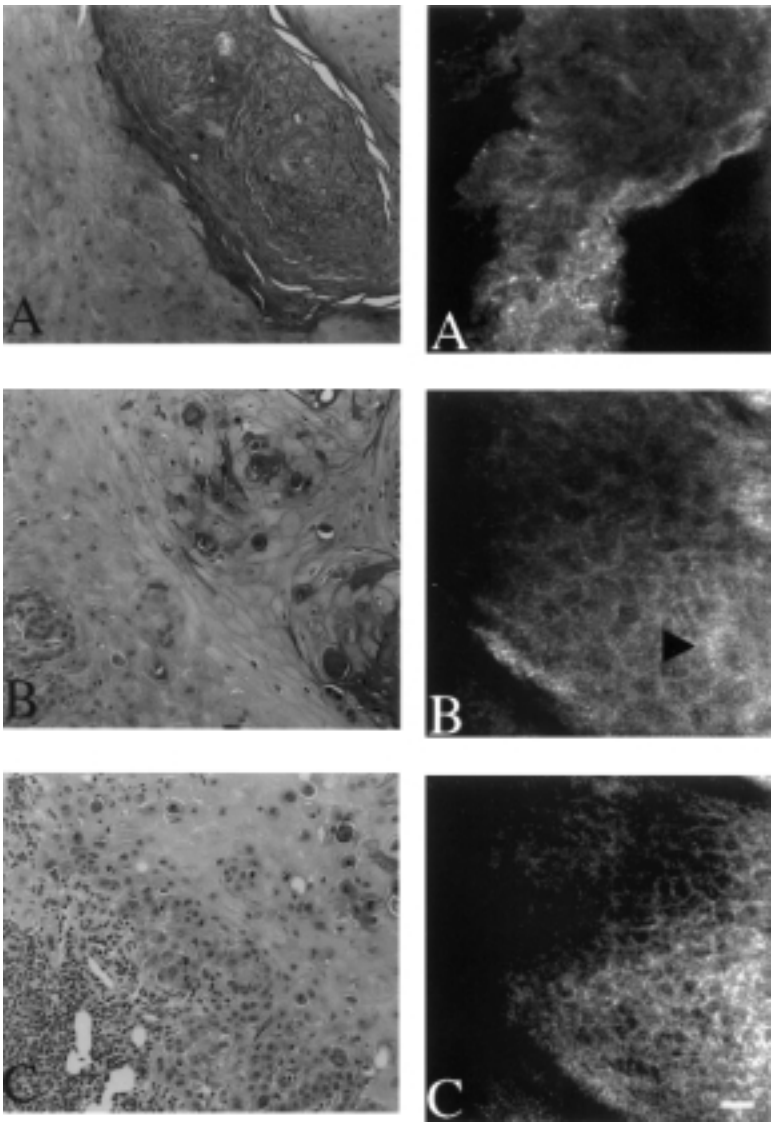

Fig 4. Horizontal sections of SCC from depths in epidermis corresponding to lettered lines in Fig 3. These were obtained using conventional histopathology (*left,* hematoxylin-eosin stain; original magnification ×10, 0.25 numerical aperture, dry objective lens) and CM (*right,* original magnification ×30, 0.9 numerical aperture, water immersion objective lens, scale bar = 25 μm). **A,** Stratum corneum. Thick, irregular hyperkeratosis evident on both conventional histopathology and CM. **B,** Stratum granulosum. Conventional histopathology reveals premature keratinization and obvious architectural disarray, features that appear more subtle on CM. A dyskeratotic cell was identified on CM (*arrowhead*). **C,** Stratum spinosum. Both conventional histopathology and CM show enlarged, pleomorphic nuclei with haphazard orientation. Dyskeratosis and an inflammatory infiltrate are evident on conventional histopathology.

inates in the basal layer and moves upward, whereas in SCC, invasion proceeds from the stratum basale downward through the dermoepidermal junction. The inability of CM to image to this level in potentially malignant hyperkeratotic lesions excludes important information from the diagnostic process. In one patient in this study, CM was barely able to distinguish an SCC that was clearly evident with traditional histopathology by virtue of invasion through the dermoepidermal junction. Instead, CM must rely on the fact that cytologic atypia in SCC involves the full thickness of the epidermis, including the stratum granulosum, which can be imaged easily in all but the most hyperkeratotic lesions. In retrospect, this SCC in our study was distinguished by slight nuclear enlargement

and pleomorphism in the stratum granulosum. However, although granular nuclear enlargement and pleomorphism support the diagnosis of invasive SCC, the distinction between invasive SCC and SCC in situ or bowenoid AK is impossible without visualizing the dermoepidermal junction.

Despite these limitations, CM may become an alternative to biopsy in the diagnosis of AKs. In the future, advances in the technology of CM may improve its optical penetration depth. Moreover, the laser power could be increased to intensify imaging at deeper levels. The refractive index mismatch between skin (1.34) and water (1.33) or gel (1.3335) can be improved by devising more optimal immersion media. The irregular surface of hyperkeratotic skin and air trapped between scales also represent hindrances to imaging that can be addressed. A solution with the appropriate refractive index and adequate diffusion properties can be applied to fill in the air pockets that disrupt imaging. Alternatively, removal or dissolution of the hyperkeratotic stratum corneum could facilitate more complete imaging of the epidermis. Notwithstanding, this preliminary study demonstrates the ability of CM to distinguish pathologic features of nuclear enlargement, nuclear pleomorphism, and architectural disarray that characterize epidermal neoplasms.

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REFERENCES

1. Schwartz RA. Premalignant keratinocytic neoplasms. J Am Acad Dermatol 1996;35:223-42.
2. Drake LA, Ceilley RI, Cornelison RL, Dobes WL, Dorner W, Goltz RW, et al. Guidelines for care of actinic keratoses. J Am Acad Dermatol 1995;32:95-8.
3. Schwartz RA. The actinic keratosis: a perspective and update. Dermatol Surg 1997;23:1009-19.
4. Mittelbronn MA, Mullins DL, Ramos-Caro FA, Flowers FP. Frequency of pre-existing actinic keratoses in cutaneous squamous cell carcinoma. Int J Dermatol 1998;37:677-81.
5. Callen JP, Bickers DR, Moy RL. Actinic keratoses. J Am Acad Dermatol 1997;36:650-3.
6. Whited JD, Horner RD, Hall RP, Simel DL. The influence of history on interobserver agreement for diagnosing actinic keratoses and malignant skin lesions. J Am Acad Dermatol 1995;33:603-7.
7. Pierard JE. In vivo confocal microscopy: a new paradigm in dermatology. Dermatology 1993;186:4-5.
8. Pawley JB, editor. Handbook of biological confocal microscopy. 2nd ed. New York: Plenum Press; 1995.
9. Rajadhyaksha M, Grossman M, Webb R, Anderson RR. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. J Invest Dermatol 1995;104:946-52.
10. Webb RH. Confocal optical microscopy. Rep Prog Phys 1996;59: 427-71.
11. 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.