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Microscopy with ultraviolet surface excitation (MUSE): A novel approach to real-time inexpensive slide-free dermatopathology

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Abstract

Traditional histology relies on processing and physically sectioning either frozen or formalinfixed paraffin-embedded (FFPE) tissue into thin slices (typically 4–6 μm) prior to staining and viewing on a standard wide-field microscope. Microscopy using ultraviolet (UV) surface excitation (MUSE) represents a novel alternative microscopy method that works with UV excitation using oblique cis-illumination, which can generate high-quality images from the cut surface of fresh or fixed tissue after brief staining, with no requirement for fixation, embedding and histological sectioning of tissue specimens. We examined its potential utility in dermatopathology. Concordance between MUSE images and hematoxylin and eosin (H&E) slides was assessed by the scoring of MUSE images on their suitability for identifying 10 selected epidermal and dermal structures obtained from minimally fixed tissue, including stratum corneum, stratum granulosum, stratum spinosum, stratum basale, nerve, vasculature, collagen and elastin, sweat glands, adipose tissue and inflammatory cells, as well as 4 cases of basal cell carcinoma and 1 case of pseudoxanthoma elasticum deparaffinized out of histology blocks. Our results indicate that MUSE can identify nearly all normal skin structures seen on routine H&E as well as some histopathologic features, and appears promising as a fast, reliable and cost-effective diagnostic approach in dermatopathology.

Keywords

MUSE; ex-vivo microscopy; dermatopathology; ultraviolet surface excitation

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Conflict of interest

Dr. Levenson is co-founder and CEO of MUSE Microscopy Inc., a Del-aware Corporation, a start-up that intends to commercialize this technology. The remaining authors declare that they have no conflicts of interest concerning this article.

1 | INTRODUCTION

Ex vivo microscopy (EVM) represents an emerging field in which various methods are employed to generate imaging of lesional tissue without processing or histological sectioning of paraffin blocks (thinsectioning). A number of technologies are under development, including multiphoton confocal imaging,[1] and optical coherence tomography.[2] These methods typically require complicated and expensive instrumentation, have limited commercial availability, and may require significant postprocessing of acquired images. Here, we evaluate the use of a new ex vivo imaging modality, microscopy using ultraviolet (UV) surface excitation (MUSE), to provide diagnostic-grade images that are comparable to those obtained from traditional formalin-fixed paraffin-embedded (FFPE) processing and H&E staining in dermatopathology.[3] A key feature of the MUSE technique is the narrow depth of penetration of UV excitation radiation, which effectively limits the excitation volume to a thin layer near the surface of the tissue.[4] This removes the requirement for physical thin-sectioning of the tissue and thus speeds up the overall microscopy process by eliminating nearly all of the preparation steps required for conventional histopathology (fixation, overnight tissue processing, paraffin embedding, thin-sectioning, H&E staining, cover slipping).[5] This method has been previously reported for the detection of endogenous fluorescent molecules in intact human tissues for functional and structural characterization, with examples documenting real-time microscopic imaging of esophageal epithelial disease[6] and noninvasive detection of Barrett's esophagus and dysplasia.[7] Our study compares MUSE microscopy with conventional H&E in dermatopathology, which has not been done so far.

2 | MATERIALS AND METHODS

MUSE microscopy employs excitation radiation provided by UV-emitting light emitting diodes (LEDs) with maximum emission wavelength centered between 275 and 285 nm. The LED output is focused using a ball lens with a short focal length onto the flat or cut surface of a briefly stained sample (see below) at an oblique angle (approximately 30°, avoiding the need for UV-transparent objectives or dichroic mirrors).

The UV radiation penetrates to a depth of 6 to 8 μm (slightly thicker than a conventional slide) from the tissue surface and excites the red. The excited light is collected by a ×10 objective under Z-axis control and focused via a tube lens onto a high-resolution color camera to generate a full-color image of the tissue surface. The setup is equipped with an XY stage for positioning of the tissue for capturing of larger areas by mosaicking series of adjacent frames, giving the observer/pathologist the ability to visually inspect the entire tissue surface (Figure 1).

This study was performed for validation of MUSE microscopy and was granted Institutional Review Board exemption at UC Davis (IRB ID# 743439-1). The specimens were selected from de-identified skin excision samples received by the dermatopathology lab during one service day. Aiming to acquire normal skin, we selected samples from specimens with the size equal to or greater than 1 cm in greatest dimension (ranging from 1.0 cm to 2.5 cm) with at least 0.3 cm grossly normal skin at the periphery. Six de-identified skin samples were

cho- sen and grossed into 3-mm-thick slices by a histotechnologist (traditional grossing). These samples were transferred into the grossing room in formalin containers and were minimally fixed (formalin exposure time varied between 15 minutes and an hour, therefore the cut surfaces of the specimens after grossing were not grossly fixed by formalin). Normal-appearing slices of each sample were stained with 4 commercially available staining agents (rhodamine B, Hoechst 33342, eosin and propidium iodide) for 40 seconds followed by washing for 10 seconds in phosphate-buffered saline (PBS). Then, 50 MUSE images were captured from different parts of the normal skin samples. In order to have the same brightness and color density among all images, MUSE images were white-balanced using an open-source image editing program (GNU Image Manipulation Program [GIMP]). After MUSE imaging, the samples were returned to formalin and subsequently submitted for traditional paraffin-embedding and histologic sectioning. Conventional H&E-stained slides were scanned using a slide scanner (Aperio AT2, Leica Biosystems Imaging, Inc., Vista, California) for concordance. All MUSE and H&E images were examined to locate corresponding areas. Thirteen correlated paired images were identified.

We also evaluated MUSE for abnormal histology using 4 paraffin-embedded skin biopsy specimens with previously diagnosed basal cell carcinoma (BCC) and 1 paraffin-embedded skin specimen with pseudoxanthoma elasticum (PXE). The H&E slides were scanned, and subsequently, the paraffin blocks were deparaffinized, and tissue cut surfaces were stained as above and then imaged by the MUSE setup. Two similar correlated paired images of BCC were found for comparison as well as one similar correlated pair of images of PXE.

Concordance between MUSE images and H&E slides was independently evaluated by 2 pathologists-in-training (AG, S.Y.L.) and 1 experienced board-certified dermatopathologist (M.A.F.). Ten selected epidermal and dermal structures (stratum corneum, stratum granulosum, stratum spinosum, stratum basale, nerve, vasculature, collagen, and elastin, sweat glands, adipose tissue and inflammatory cells) were assessed on each pair of images by the examiners. Each MUSE image was scored from 0 to 2 per structural element based on the scoring system shown in Table 1.

3 | RESULTS

The average time needed to acquire each MUSE image was about 5 minutes (2 minutes for staining and 2–3 minutes for image acquisition), which compares favorably with typical overnight processing for H&E slides. However, the evaluators reported that interpretation of the MUSE images seemed more time-consuming than for the H&E slides, which they attributed to unfamiliarity with MUSE images. Sixteen correlated paired H&E and MUSE images from 11 patients (6 de-identified skin samples, 4 paraffin-embedded skin biopsies of BCC and 1 paraffin-embedded skin biopsy of PXE) were evaluated. Mean scores of 3 examiners for each structure were generated and shown in Table 2. The overall MUSE score of 9.4/10 indicated a slightly weaker ability to identify these normal skin structures compared to H&E. However, the comparison showed that MUSE was comparable and sometimes superior to conventional microscopy in showing certain structures such as adipose tissue, vasculature, stratum corneum and collagen and elastin fibers (Figure 2). On the other hand, MUSE depiction of cytoplasmic details was rated inferior to that of H&E, for

example, inflammatory cells and stratum granulosum (Figure 3). Interestingly, MUSE could clearly show diagnostic pathologic features of BCC and was also able to show the unique pattern in elastin in a patient with PXE (Figure 4).

4 | DISCUSSION

The images obtained by MUSE allowed identification of multiple skin histologic structures as well as 2 disease states (BCC and PXE) with an accuracy that approximates that of traditional H&E microscopy and showed that MUSE can be an asset in diagnosing skin pathology with- out the need of conventional H&E slides. However, evaluation of greater numbers of cases by greater numbers of observers in a wider variety of applications (eg, Mohs micrographic surgery, stat frozen sections) will be necessary to fully evaluate the potential applications of the MUSE in comparison to the traditional methods.

MUSE can generate images in just a few seconds after 2 to 3 minutes of staining compared to the traditional overnight processing that requires several hours for H&E slides to be prepared. Thus, MUSE images have potential use in “point-of-care” (POC) settings with applications in intraoperative surgical pathology and both urgent and routine outpatient evaluation and management. In the latter cases, patients could be notified of biopsy results during their clinic visit. The stains used for MUSE are commercially available and no more expensive than H&E stains. The overall costs are even lower, considering that significant additional labor, material and disposal expenses are required to prepare H&E slides. Medicare currently reimburses $32.65 to prepare 1 H&E slide (Medicare Fee Schedule for CPT 88305-TC, MAC locality 0111263; https://www.cms.gov/apps/physician-fee-schedule/search/search-results.aspx?Y=0&T=0&HT=0&CT=2&H1=88305&C=18&M=4, accessed February 24, 2018). The MUSE setup used in this study was a prototype and may be subject to change in the future, so the actual cost of the hardware, software and licensed reagents cannot be exactly predicted at this point. However, it should be well within most pathology departments’ budgets. The author most closely involved with operational aspects of MUSE (R.L.) is confident that MUSE would ultimately be less expensive, overall, than conventional histology.

After MUSE imaging, the specimens can be fixed, embedded and stained conventionally without evidence of interference with specimen quality and staining results to date. As MUSE is non-destructive and does not require fixation, the specimen may potentially be used for subsequent molecular studies with enhanced molecular analyte recovery compared to that achievable with FFPEbased methods.

A potential limitation is that MUSE images are currently limited to ×10 magnification (while H&E slides can be scanned at ×40 magnification). This may explain why MUSE showed a lower ability to reveal cytoplasmic and nuclear details compared to H&E, and in this study was less capable of identifying inflammatory cells and the stratum granulosum. However, techniques and material used for sample preparation as well as instrument optical design continue to be optimized, which may potentially overcome these limitations.

Another limitation of our study was due to the difference in methodology in acquiring MUSE images and H&E slides, which made it difficult to obtain perfectly paired correlated images for comparison; MUSE images were taken from the cut surface of the specimen. H&E slides were mostly from slightly deeper levels of the skin due to the need to remount and face the blocks. This made it difficult or, in some cases impossible, to find a perfectly correlated image from the same spot and same level for comparison. For that reason, the number of evaluated paired images in this study was limited to 16.

Comparison of MUSE and H&E slides showed that distinguishing an adipocyte from artifact or vessel was not completely straightforward with H&E-stained slides, but MUSE was able to show distinct surface topology of the adipocyte globules and vascular tubal structures (Figure 2). Collagen and elastin structures were also highlighted by a green or blue coloration, respectively, in MUSE images, which helped to identify these structures more easily than the pink variants visualized with H&E.

5 | CONCLUSION

MUSE represents an emerging technology for rapid diagnostic imaging of skin pathology directly on excised tissue in the clinic, without the need for tissue fixation, processing or thin-sectioning. This study was limited, but it showed that MUSE represents a novel EVM method that is cost-effective, resource-friendly, and fast; even at this stage of development it appears to generate diagnostic-quality images, at least for some lesions. As it is non-destructive, if results are non-diagnostic, the specimen can be submitted for routine histology without impact on conventional clinical workflow. MUSE has potential to be serve in “POC” settings for evaluating surgical pathology specimens at both intraoperative (such as Mohs micrographic surgery) and routine outpatient evaluation and management by providing biopsy results during a patient’s clinic visit. More study is required to compare MUSE with traditional methods and determine whether it can be validated and accepted for patient care.

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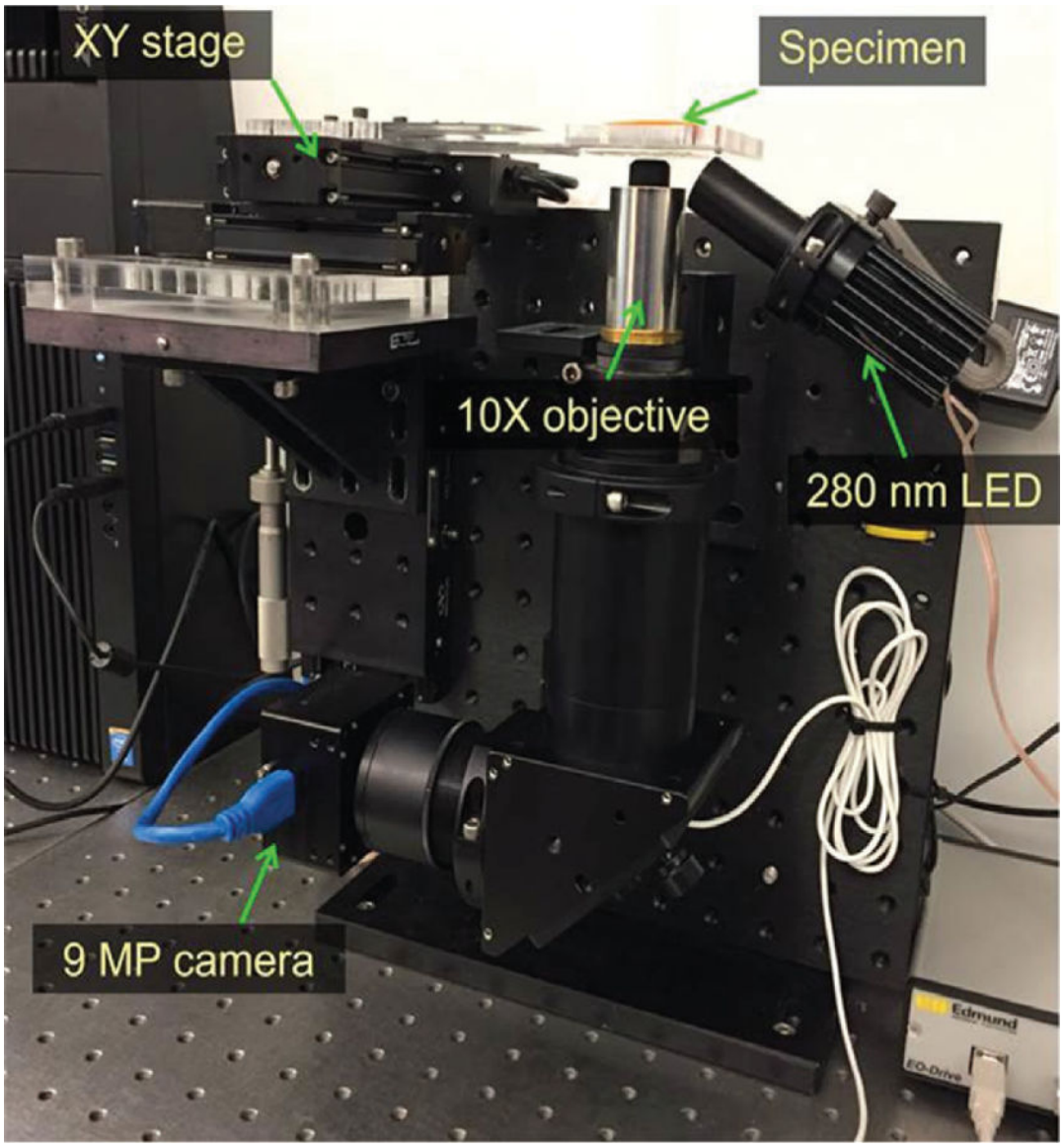

FIGURE 1.

Microscopy using ultraviolet surface excitation (MUSE) microscope setup. A light emitting diode (LED) emits 280-nm ultraviolet (UV) radiation that excites the stained tissue surface to generate visible emissions. The excited light is collected by a ×10 objective, focused via a tube lens onto a 9 megapixel camera. 666 × 718 mm (300 dpi)

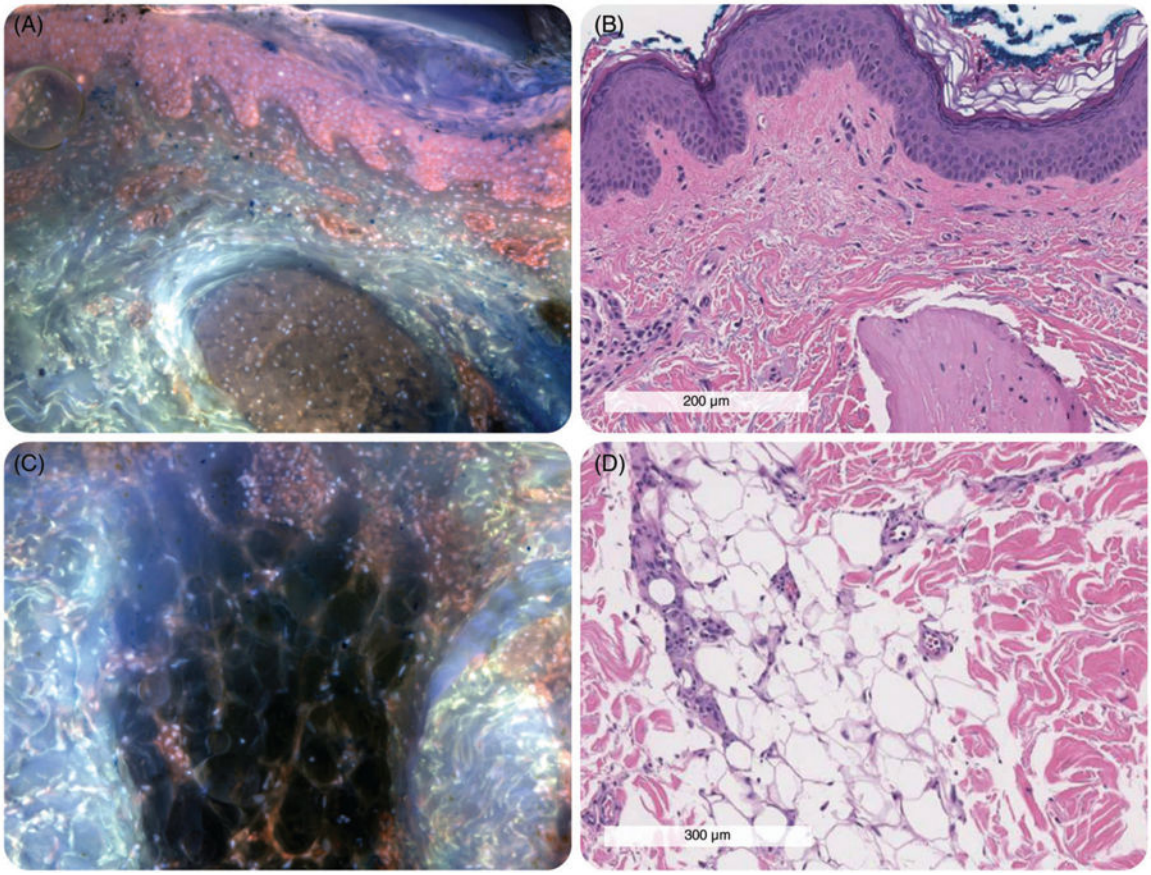

FIGURE 2.

Skin samples with minimal formalin exposure: (A) microscopy using ultraviolet surface excitation (MUSE) shows vascular structures visible as brown hollow structures. The arrector pili is gray-brown. Collagen and elastin fibers around the arrector pili are highlighted blue/green. (B) H&E correlated with (A) (×150 magnification). (C) MUSE image shows fat globules in a semi-3D structure. Vessels can be seen as brown hollow structures. The collagen and elastin fibers are highlighted blue/green. (D) H&E correlated with (C) (×100 magnification)

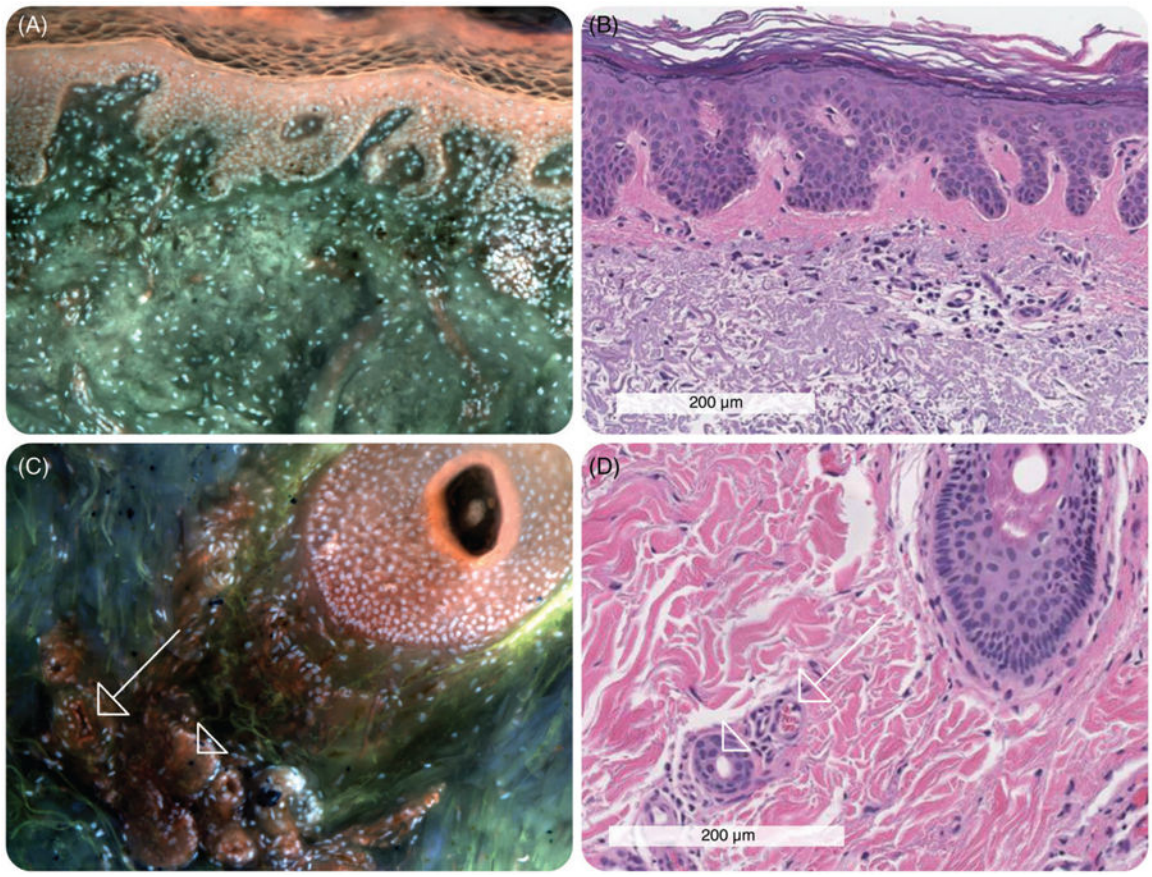

FIGURE 3.

Skin samples with minimal formalin exposure: (A) microscopy using ultraviolet surface excitation (MUSE) image does not depict keratohyaline granules in the stratum granulosum and cytologic features of inflammatory cells. The elastin fibers are faded green, possibly corresponding to the solar elastosis. (B) H&E correlated with (A) (×150 magnification). (C) MUSE image is showing a hair follicle. The collagen fibers are green. The vessels (arrow) and eccrine glands (arrow head) are tubal structures in brown. (D) H&E correlated with (C) (×165 magnification)

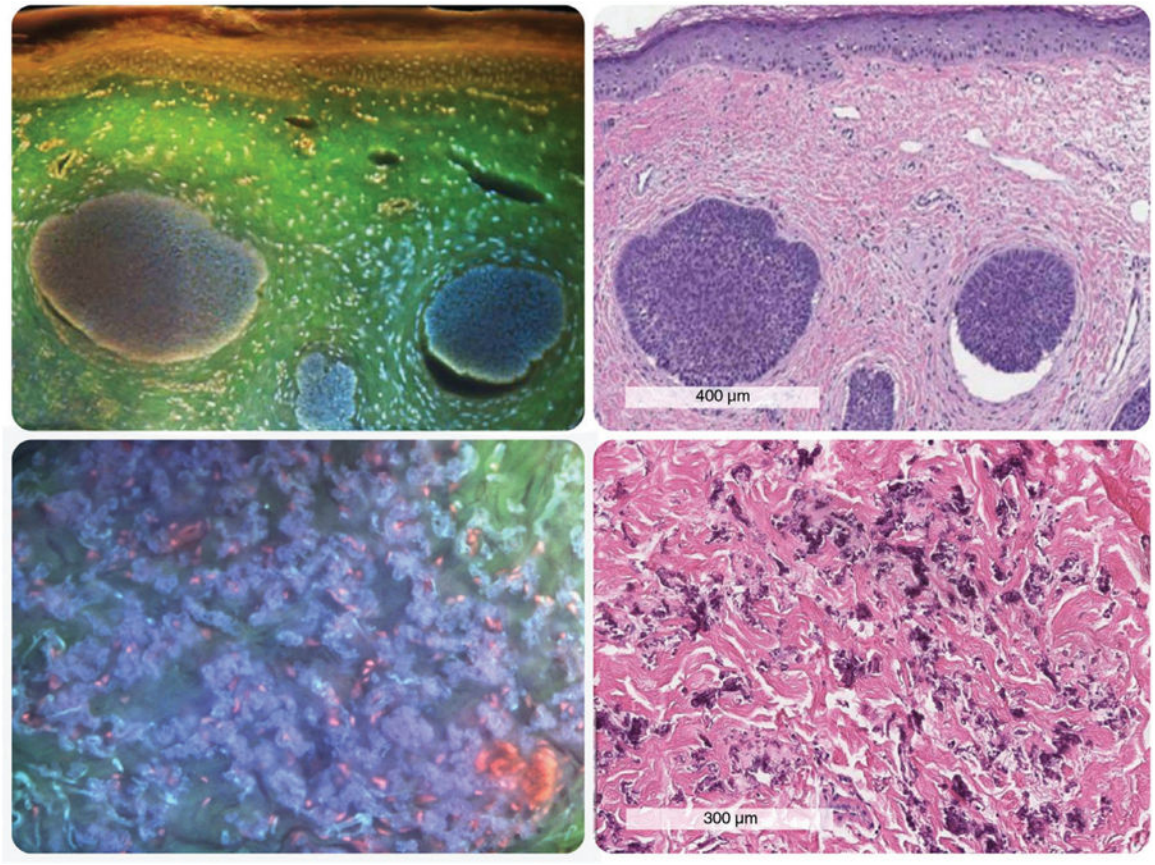

FIGURE 4.

Deparaffinized skin samples: (A) microscopy using ultraviolet surface excitation (MUSE) image shows basal cell carcinoma with peripheral palisading and tumor-stroma cleft. The collagenous stroma emissions are bright green. (B) H&E corresponding to (A) (×70 magnification). (C) Pseudoxanthoma elasticum. MUSE shows calcified, fragmented elastin (lavender) in a fluffy distribution pattern in a patient with pseudoxanthoma elasticum. (D) H&E correlated with (C) (×100 magnification)

TABLE 1

Study definitions

Score	Definition
0	MUSE cannot identify the structure without correlating with the H&E
1	MUSE can identify the structure without the need of H&E
2	MUSE can identify the structure with more certainty than H&E
N/A	Not applicable (the structure is not in the field to evaluate)

Abbreviation: MUSE, Microscopy using ultraviolet surface excitation.

TABLE 2

Comparison of normal skin structures

Dermal structures	Mean score
Stratum corneum	1.1
Stratum granulosum	0.0
Stratum spinosum	0.9
Stratum basale	1.0
Nerve	1.0
Vasculature	1.1
Collagen and elastin	1.2
Sweat glands	1.0
Adipose tissue	1.9
Inflammatory cells	0.3
Total score	9.4/10