

Noninvasive cellular imaging in live skin makes histopathology accessible for AI innovation.

Topic 1: AI to improve accuracy of diagnosis and health risk assessment.

Topic 5: Innovation in AI methods that increase AI's capacity to improve healthcare.

Background

Image classification with deep learning and convolutional neural networks (CNN) is a powerful computer vision technique with the potential to enhance medical imaging. The FDA has already cleared multiple technologies leveraging AI techniques, and more technologies are in development. While many of these technologies are intended for applications in radiology, there is substantial motivation to apply AI tools to enhance the interpretation of histopathology. Histopathology is the inspection of cellular images for atypical features of disease and is the diagnostic gold standard for many conditions. Unfortunately, for the last 150 years, cellular imaging has only been possible in tissues removed by surgical biopsies that are mounted and stained on glass slides for microscope inspection. Digitizing these slides for pairing with AI techniques adds an additional step to this already inefficient, invasive, and irreversible process¹. This inefficiency presents a substantial challenge for clinical AI as deep learning requires large curated data sets to achieve meaningful performance². AI enhanced digital pathology would benefit tremendously if there were a way to image cellular anatomy directly in the tissue without the need for surgical biopsy.

We created such a technology that images histopathology in live skin with no cuts or stains. Utilizing a custom designed portable multiphoton microscope, we generate noninvasive cellular images *in vivo* by simply touching the skin at the site of interest. Skin cancer is the most common form of human malignancy, and earlier detection is a compelling application that can benefit from AI enhanced diagnosis³. Our approach compresses a process that normally takes several days into a real time, point of care inspection where images stream at up to four frames per second. The result is an abundance of digital images containing diagnostic features that are ideal for training a CNN. The speed and ease of capturing these images enables the creation of novel, robust data sets to further improve diagnostic accuracy and expand AI access to patients normally underrepresented in biopsy-based histology such as children and people of color.

Methods

We designed a portable, battery powered, multiphoton microscope that illuminates tissue with ultrafast pulsed near infrared light that excites two-photon autofluorescence and second harmonic generation (SHG) signals in live skin. The handheld microscope makes direct contact with the skin, stabilizing the tissue and dramatically mitigating motion artifacts. SHG signals arise only in collagen fibers and the two-photon signal arises primarily in the cells⁴. By splitting these signals based on the emitted wavelengths, we create a two-color image with a “photonic stain” that closely matches the contrast generated by traditional hematoxylin and eosin staining (H&E). Our microscope images in cross section through the skin layers down to the reticular dermis in an orientation that is familiar to dermatopathologists.

We conducted *ex vivo* and *in vivo* investigations with our prototype on both healthy skin and basal cell carcinoma (BCC), which is the most prevalent form of skin cancer⁵. Our *ex vivo* investigation explored the possibility of training a CNN for identification of BCC. Our microscope can also image slides, so we ordered unstained mounted slides of BCC and healthy tissue from a specimen lab. We collected 624 images from 42 *ex vivo* samples of basal cell carcinoma and 960 images from 65 samples of healthy tissue as a preliminary training set. We further partitioned the images into 32 non-overlapping sub-regions, creating approximately 20,000 and 30,000 regions in BCC and Healthy samples, respectively.

Images were divided into the two classes for training by a practicing dermatopathologist. We reserved 8% of our images from both categories for validation. Training and testing were performed in collaboration with a software consulting firm.

Our *in vivo* imaging explored whether we could collect images in skin from diverse subjects and patients with BCC. We imaged healthy volunteers ranging in age from 5 to 85 years old with skin types II – VI in various locations on the body. We also took images in a local dermatology clinic from patients who had suspected BCC before they received a biopsy. We compared our *in vivo* images with histopathology from the biopsies to determine if we could visualize BCC non-invasively in real time. All human imaging was conducted under IRB oversight (Salus IRB) with informed patient consent.

Results

In *ex vivo* skin sections imaged with our portable microscope, the AI algorithm achieved 99% accuracy in training and 98% accuracy in validation when classifying normal and cancerous regions of samples.

In vivo images of skin displayed features of skin cancer discernable by human pathologists. *In vivo* imaging was successful in all patients, regardless of age, body location, or skin color.

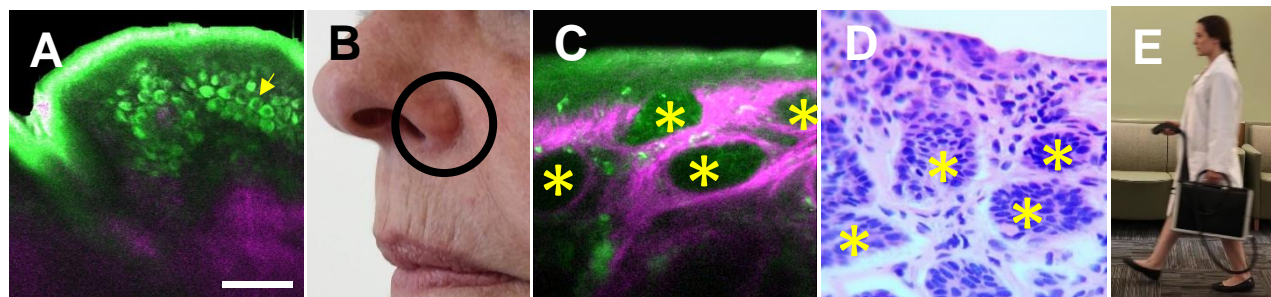


Figure 1. (a) *In vivo* cross section of live skin cells (green) and connective tissue (magenta). Cellular nuclei are visible within individual cells (yellow arrow). Scale bar: 50µm. (b) A lesion suspicious for BCC in a subject. (c) Real time *in vivo* image of suspicious lesion shows basaloid invasion indicative of BCC. (d) H&E stained image of suspicious lesion following biopsy confirms the presence of BCC. Yellow asterisks indicate correspondence between *in vivo* and *ex vivo* images. (e) Investigator carrying portable multiphoton system.

Implications for improving the value of care

In vivo histopathology yields multiple opportunities to improve AI diagnostics. We observed very strong performance in our *ex vivo* training set. Notably, we suspect the performance was bolstered by including images of health tissue in the training. Typically, dermatologists only biopsy lesions that they suspect are concerning. This is an inherent bias in the data and it is reasonable to hypothesize that including healthy skin histology in the training will boost diagnostic accuracy by exposing the algorithm to a broader range of what is considered normal histology. While it would be impractical to widely biopsy healthy individuals solely to create a sizeable training set, we can generate these data rapidly and non-invasively by imaging healthy volunteers. Moreover, we can be more inclusive in our imaging and recruit subjects with darker skin who less frequently visit the dermatologist and for which there is a dearth of representation in current histology specimen banks. With our rapid streaming (240 images/minute) and ability to scan across the skin, we generate a multitude of similar though non-identical images of the underlying histology that creates natural data augmentation for more robust training. Finally, as a completely non-invasive technique, our approach could enable longitudinal monitoring of suspected cancer for the first time. It may be possible guide the dosage and location of application of emerging topical cancer treatments which would elevate histology into the realm of therapy.

References

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