Biopsy-free Virtual Histology of Skin Using Reflectance Confocal Microscopy and Deep Learning

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Abstract: We report *in vivo* virtual histology of skin without a biopsy, where deep learning is used to virtually stain tissue and generate hematoxylin and eosin (H&E)-like microscopic images of skin using a reflectance confocal microscope.

1. Introduction

Skin cancer is the most prevalent type of cancer diagnosed worldwide. In general, an invasive biopsy and histological staining of tissue are required for traditional pathological identification of skin cancer, which are time-consuming and inefficient, often resulting in unnecessary scarring. As one of the emerging noninvasive optical technologies for skin disease diagnosis, reflectance confocal microscopy (RCM) forms a biopsy-free solution to provide *in vivo* images of the skin structure with cellular-level resolution. However, evaluating these RCM images requires specialist expertise, due to fact that these images are grayscale, lack nuclear contrast, and have a limited association with histology.

In recent years, the emergence of deep learning provides a promising route for computer-assisted diagnosis using label-free tissue images. Many efforts have been invested along this direction to achieve rapid skin disease identification, such as employing deep neural networks to achieve the classification of skin cancer using photographs of skin [1] and segmentation of cellular patterns based on RCM images of melanocytic lesions [2]. Deep learning-based approaches were also investigated for virtual staining of tissue through image transformations performed between two different microscopy modalities. For example, a convolutional neural network can be used to transform the autofluorescence or phase images of label-free tissue sections into virtually stained images, which are indiscernible to pathologists compared with their histochemically stained counterparts [3–5]. However, these virtual staining approaches still rely on the excision and thin sectioning of tissue.

Here, we report non-invasive, biopsy-free virtual histology of skin, based on *in vivo* label-free RCM images. Using a generative adversarial scheme, we trained convolutional neural networks to rapidly transform *in vivo* RCM images of unstained skin into virtually-stained H&E-like 3D images with microscopic resolution [6]. Our virtual histology framework bypasses both tissue biopsy and histochemical staining processes, and successfully produces results with features consistent with histochemically stained counterpart images obtained from the same excised tissue.

2. Results and Discussion

First we started with using *ex vivo* RCM image stacks of excised unstained tissue as inputs and RCM image stacks of the same tissue stained with acetic acid as ground truth (see Fig. 1); this initial step helped us create an acetic acid virtual staining neural network named VS_{AA} which was trained to generate nuclear contrast for *in vivo* RCM images of skin [6]. Then, these acetic acid virtual staining results were further transformed into H&E-like images using a second trained deep neural network, named as the H&E virtual staining network or VS_{HE}. As shown in Fig. 2, we demonstrated that this framework is applicable to various conditions, including normal skin, basal cell carcinoma, and melanocytic nevi with pigmented melanocytes. This 3D virtual staining framework can be also applied for different skin layers, including epidermis, dermal-epidermal junction, and superficial dermis layers. We quantified the success of our *ex vivo* acetic acid virtual staining results by analyzing the sensitivity and precision of nuclear predictions and calculating a set of nuclear morphological metrics (e.g., nuclear size, eccentricity, compactness, contrast, and concentration), which presented a very good match with those calculated based on the actual acetic acid-stained ground truth images [6]. Our *in vivo* virtual staining results were also evaluated by comparing them to traditional histochemically stained counterparts excised from the same tissue, demonstrating histological characteristics similar to standard H&E results [6]. We believe that *in vivo* virtual staining of unstained skin through non-invasive RCM imaging can open a unique route to rapid and accurate diagnosis of malignant skin neoplasms, which may reduce the need for unnecessary skin biopsies in the clinics.

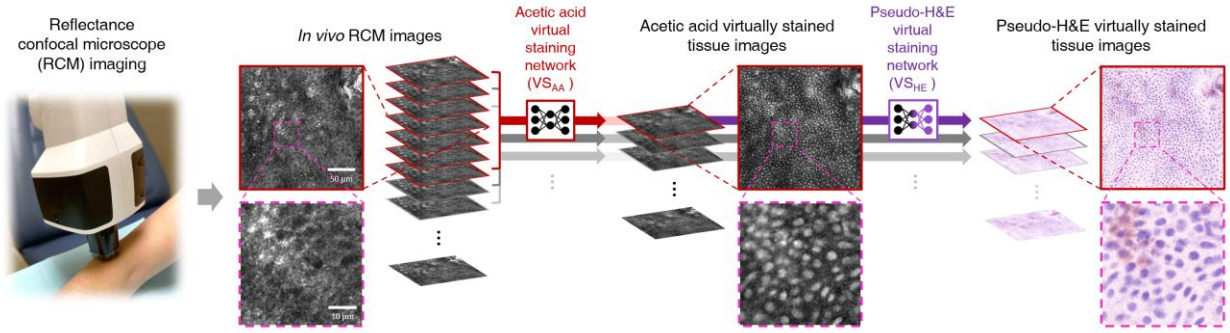

Fig. 1. The workflow of biopsy-free virtual histological staining for skin pathology. After biopsy-free skin imaging using RCM, for each virtual staining inference, seven RCM images corresponding to axially adjacent planes within skin tissue are fed into a trained deep neural network VS_{AA}, which transforms these images into an acetic acid virtually stained tissue image that is corresponding to the central plane of the input image stack. Following this acetic acid virtual staining, another trained deep neural network (VS_{HE}) is successively used to perform H&E virtual staining through VS_{HE} [6].

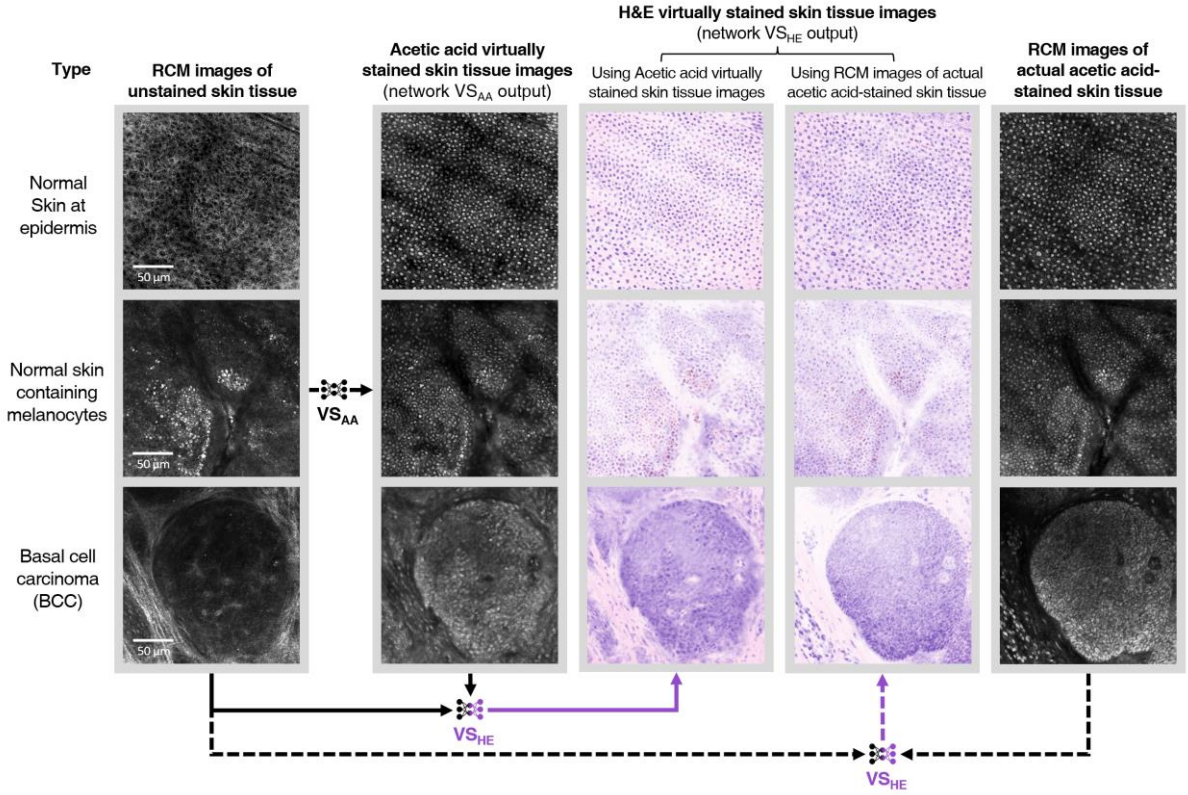

Fig. 2. Virtual acetic acid and H&E staining results for normal skin (1st row), a melanocytic nevus with pigmented melanocytes (2nd row), and basal cell carcinoma (3rd row), along with their comparison with actual acetic acid staining ground truth [6].

3. References

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