Deep Learning Enables Non-invasive, Biopsy-free Virtual Histology of Skin Using Reflectance Confocal Microscopy

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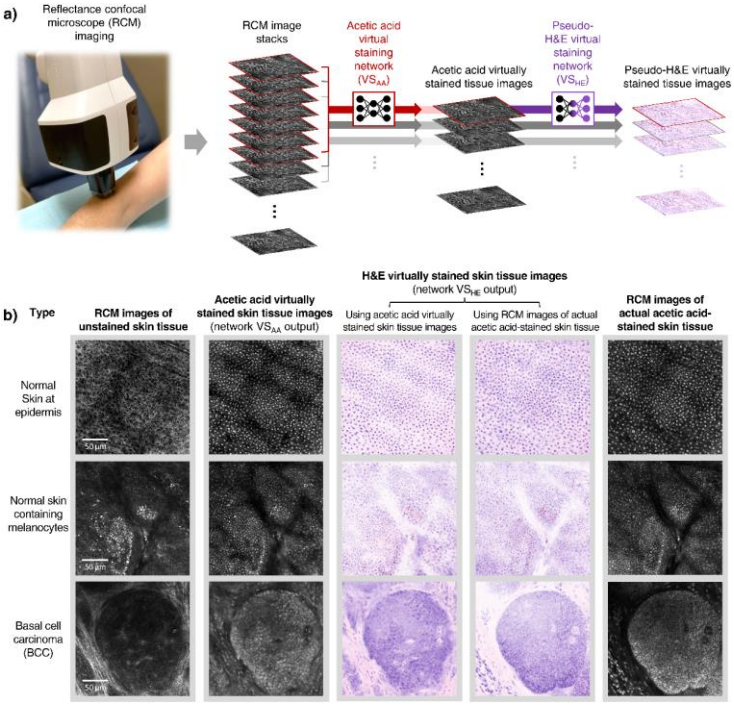
Introduction: Traditional pathological evaluation of skin cancer requires an invasive biopsy of skin tissue, followed by sectioning and histochemical staining. Despite being the gold standard in the clinic, this process is time-consuming and often results in unnecessary scarring. Reflectance confocal microscopy (RCM) is an emerging non-invasive optical imaging technique, which can provide *in vivo* images of skin tissue structure with a cellular-level resolution, forming a biopsy-free solution for skin disease diagnosis. However, interpreting the resulting images from RCM requires specialized training since they are grayscale, lack nuclear contrast, and have a limited correlation with histology. To break this dilemma, here we leverage deep learning to develop a virtual staining method that can perform non-invasive virtual histology of skin based on *in vivo*, label-free RCM images [1].

Materials and Methods: Using RCM images of excised unstained skin tissue samples as inputs and RCM images of the same tissue stained with acetic acid to generate nuclear contrast as ground truth, we trained a convolutional neural network (CNN) named acetic acid virtual staining network (VS_{AA}), which rapidly transforms *in vivo* RCM images of unstained skin into virtually acetic acid-stained tissue images, as illustrated in Fig. 1a [1]. Then, a second deep neural network named H&E virtual staining network (VS_{HE}) is trained to transform these acetic acid virtual staining results into H&E-like images [1].

Results and Discussion: As shown in Fig. 1b, we demonstrated that this framework is applicable to various types of skin tissues, including normal skin, basal cell carcinoma, and melanocytic nevi with pigmented melanocytes [1]. This 3D virtual staining framework can also be applied to different skin layers, such as the epidermis, dermal-epidermal junction, and superficial dermis. We confirmed the success of our *ex vivo* acetic acid virtual staining results by calculating the sensitivity/precision of nuclear predictions and measuring the nuclear morphological parameters, which presented a good match with those calculated based on the actual acetic acid-stained ground truth images. Our *in vivo* virtual staining results were also compared to conventional histochemically stained counterparts excised from the same tissue, revealing histological features similar to standard H&E histology.

Figure 1. a) The schematic diagram of biopsy-free virtual histological staining procedures for skin pathology. For each virtual staining inference, a stack of seven axially adjacent RCM images of skin tissue is fed into a trained deep neural network VS_{AA} and transformed into an acetic acid virtually stained tissue image corresponding to the central plane of the input image stack. Following this step, another trained deep neural network, VS_{HE}, is used to perform pseudo-H&E virtual staining based on the acetic acid virtually stained tissue images. [1] **b)** Virtual acetic acid and H&E staining results for different types of skin structures, along with their comparison with actual acetic acid staining ground truth.



Conclusions: The reported skin virtual histology framework can transform RCM images of the skin tissue into H&E-like, virtually stained tissue images similar to histochemically stained counterparts excised from the same sample, and this unique capability may open new avenues to biopsy-free, label-free clinical dermatological diagnosis.

Reference: [1] Li J, *et al.* Biopsy-free *in vivo* virtual histology of skin using deep learning. *Light Sci Appl* 2021; **10**: 233.