Deep Learning-Enabled Virtual H&E and Fluoro-Jade B Tissue Staining for Neuronal Degeneration

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1 INTRODUCTION

In nonclinical safety assessment, pathologists routinely evaluate brain sections for neuronal degeneration indicative of neurotoxicity. Conventionally, this consists of microscopic examination of brain sections that have been stained with hematoxylin and eosin (H&E). Additional brain sections can be stained with a fluorochrome such as Fluoro-Jade B (FJB), in which degenerate neuronal cell bodies appear bright green using a fluorescein filter. Neurotoxicity studies often include an expanded neuropathologic examination of both H&E and FJB sections which can substantially increase the time and cost. Here we present a deep learning-based framework applied to unstained brain sections to create virtual H&E and FJB images. Efficacy was assessed using a kainic acid-treated rat model of neuronal degeneration.

2 MATERIALS & METHODS

Whole slide images of 31 unstained brain sections were captured with a fluorescence scanning microscope (Zeiss Axio Scan.Z1) using multiple fluorescence filter sets, (e.g., DAPI, EGFP, Cy3). To train the virtual FJB model, sample sections were then stained with FJB and scanned with the EGFP filter to create the training labels. The corresponding unstained and FJB-stained field of view within each sample were then accurately co-registered, of which 29 sections were used as the training set, 1 independent section was used as the validation set (for model selection), and 1 independent section was used for blind testing. 23 of the sections were subsequently stained with H&E and the process was repeated using the same validation and testing slides to create the virtual H&E staining model. The deep neural network models were built based on U-net architecture and trained for ~2 days on a computer equipped with 2 NVIDIA RTX 3090 graphics cards until convergence (Figure 1).

3 RESULTS

Application of each virtual staining model to the digitized label free tissue section resulted in essential equivalence between the virtual and histochemical-stained sections (Figure 2). This technology offers multiple virtually stained images from a single unstained section, reducing the time, labor, and reagents associated with slide staining. Additionally, this technique allows for staining colocalization of structures and could lead to more frequent FJB assessments. Research is ongoing to create additional virtual histochemical and immunohistochemical stains of interest for expanded neuropathologic assessments.

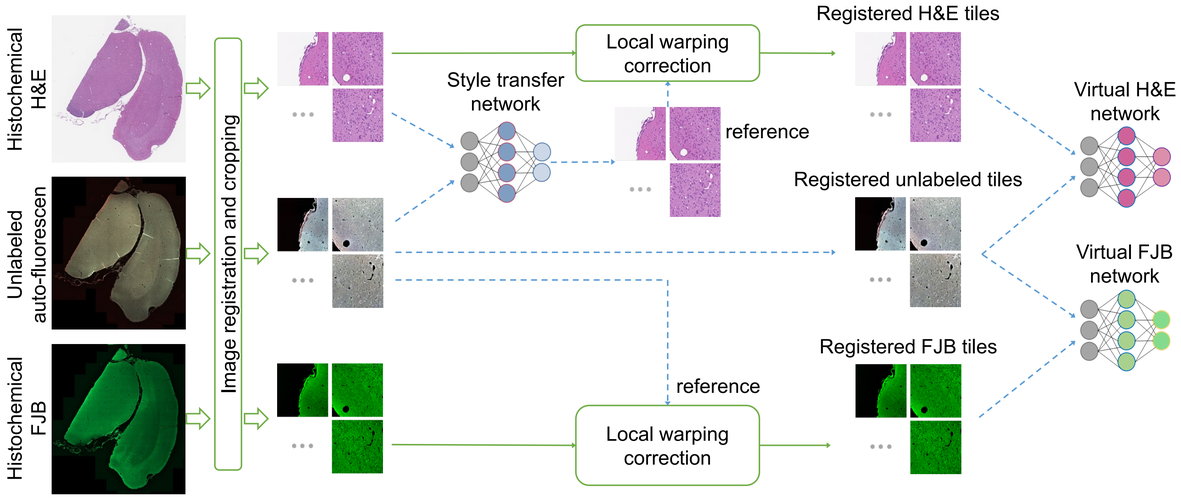

Figure 1. Fluoro-Jade B and H&E virtual staining deep network training workflow.

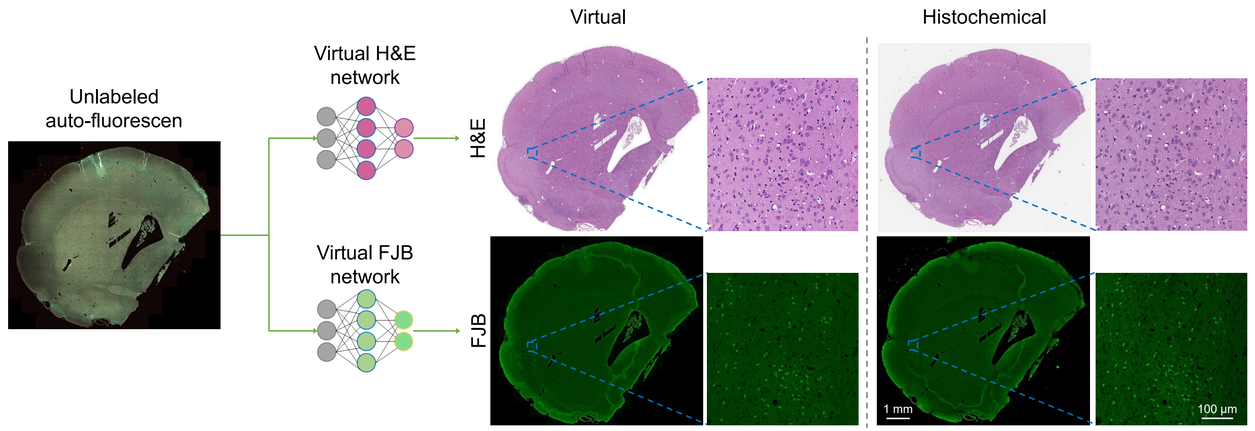

Figure 2. Virtually Fluoro-Jade B and H&E virtual staining performed on the same label-free tissue section, compared with the corresponding histochemical staining.