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VirtualPNN: Deep Learning for Label-free Visualization of Perineuronal Nets in Brain Tissue

Vishva Natarajan, MS[1], Tanmay Shukla, MS[1], Naofumi Tomita, MS[1], Scott Palisoul, BS[2], Madhumala Sadanandappa, PhD[2], George Zanazzi, MD, PhD[2], Jennifer Hong, MD[3], and Saeed Hassanpour, PhD[1]

[1]Geisel School of Medicine at Dartmouth, [2]Department of Pathology and Laboratory Medicine, DHMC,[3]Department of Neurosurgery, DHMC

Introduction

Brain-tumor related epilepsy (BTRE) is a leading cause of morbidity in patients with brain tumors.

Emerging evidence indicates **Perineuronal Nets (PNNs)**, extracellular matrix proteins that surround inhibitory interneurons, may modulate BTRE.

Immunohistochemistry (IHC) is used to detect the presence of PNNs in brain tissue. However, IHC staining is tissue-destructive and costly.

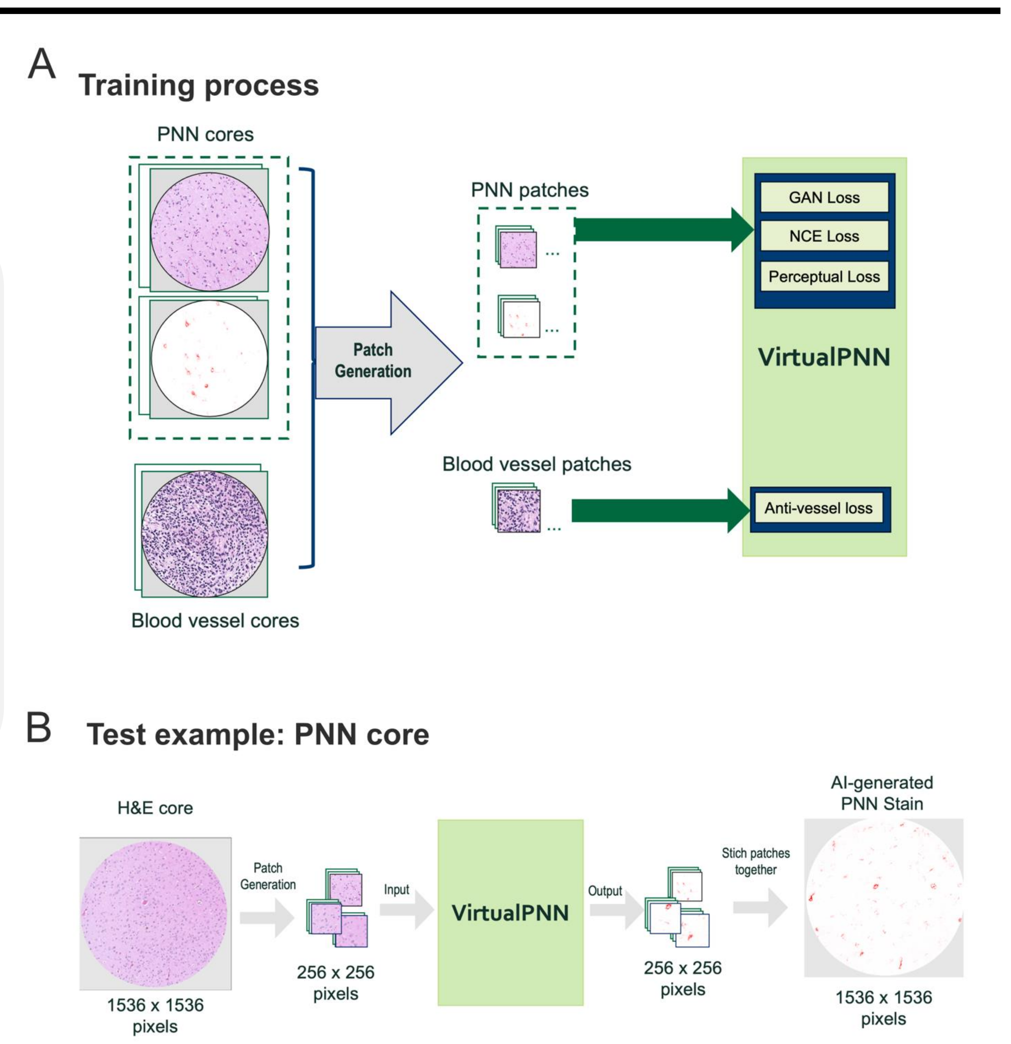
Example of brain tissue that was destroyed during IHC process.

Simulated IHC staining using generative AI, called **virtual staining**, can computationally re-create laboratory IHC and fluorescence staining patterns given an input H&E stain.

We introduce **VirtualPNN:** a generative AI tool to visualize PNNs in brain tissue without physically staining it.

Methods

Figure 1. Training and testing overview of VirtualPNN.
(A) For PNN cores, paired H&E and aggrecan stains are used; for vessel cores, only H&E is provided. VirtualPNN is trained on PNN patches with three objectives: GAN loss (realistic appearance), contrastive loss (H&E–aggrecan feature alignment), and perceptual loss (fine mesh texture). Vessel patches drive the anti-vessel loss, suppressing false-positive staining. Together, these guide VirtualPNN to generate realistic, biologically faithful PNN stains.



High-Fidelity Virtual Histopathology

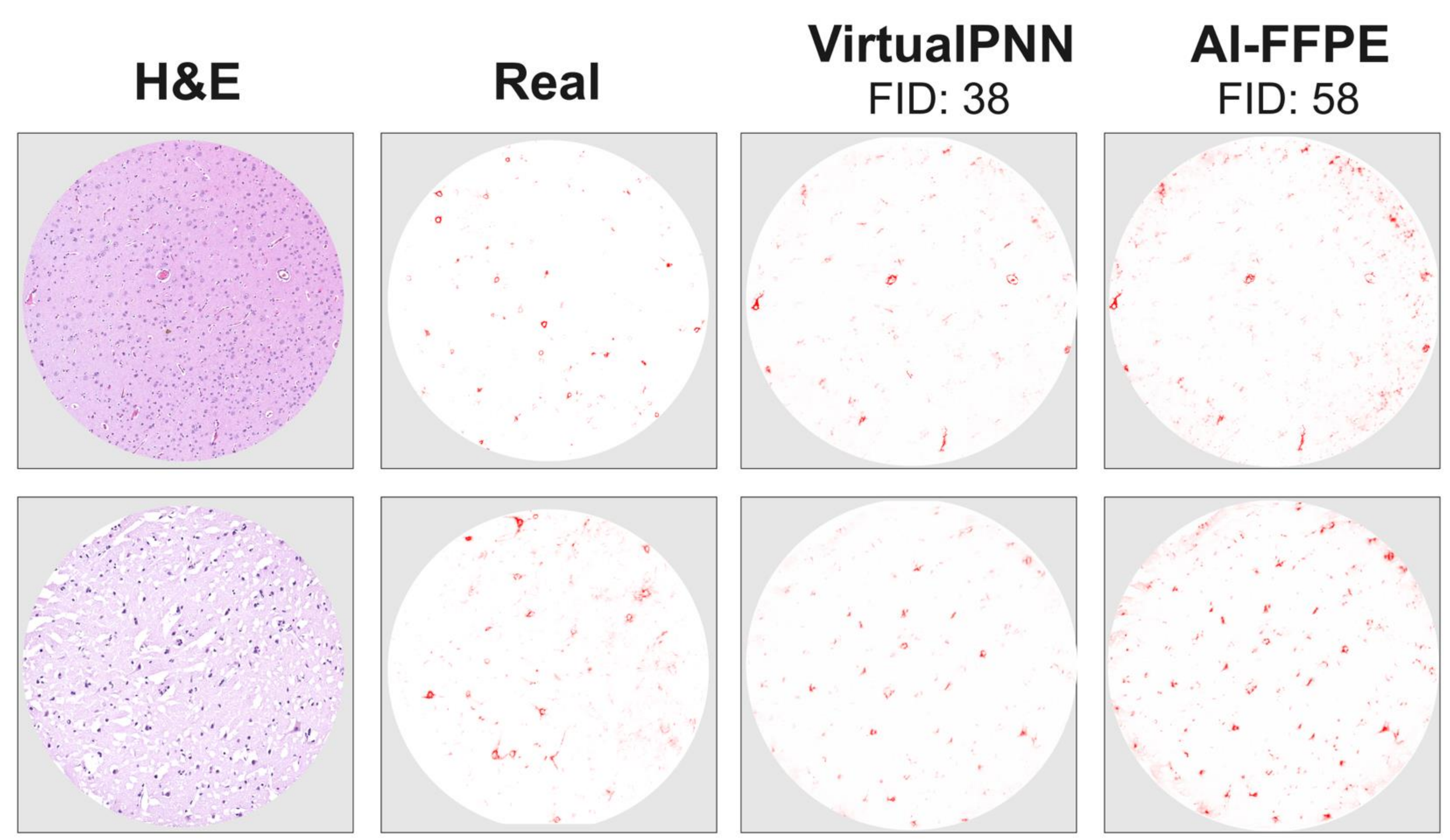
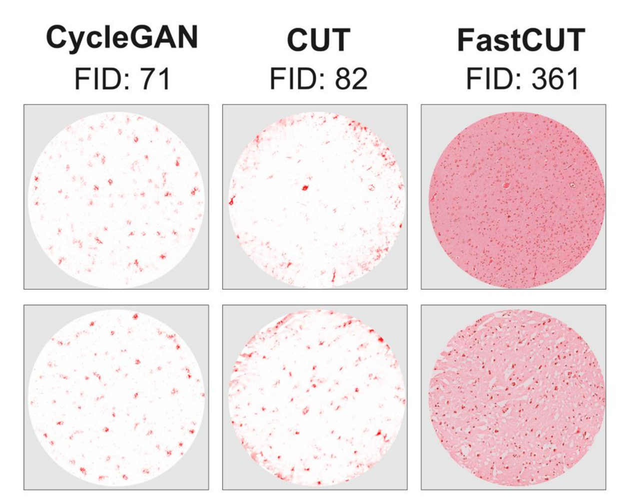


Figure 2. Benchmarking VirtualPNN against other leading virtual-staining architectures. Example H&E input with real vs. AI-generated stains. Image quality was assessed using Fréchet Inception Distance (FID; lower = better). After fine-tuning each model on aggrecan IHC, VirtualPNN achieved the best performance (FID = 38), outperforming AI-FFPE (58), CycleGAN (71), CUT (82), and FastCUT (361)



Validation by Neuropathologists

We administered a **Visual Turing Test**, tasking 2 board-certified Neuropathologists to distinguish real (N=20) from AI-generated (N=20, 5 from each model) images. All images were randomized.

Figure 3. VirtualPNN outperformed competitor models AI-FFPE, CUT, and Cycle GAN in fooling rates, defined as the proportion of real patches predicted to be AI-generated.

VirtualPNN Avoids Staining Blood Vessels

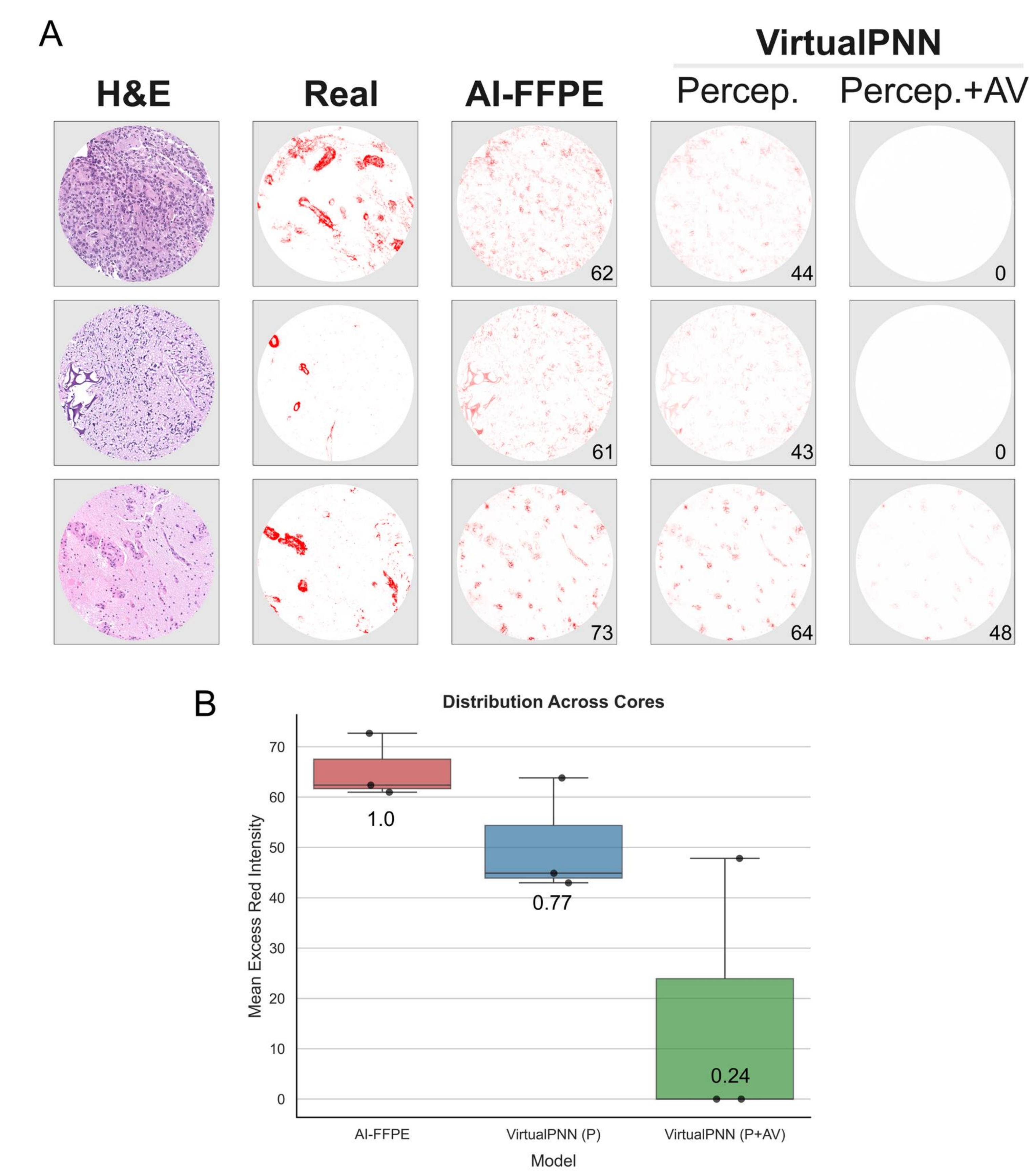


Figure 3. Anti-vessel loss reduces false-positive vessel staining.
(A) In vessel-only cores, AI-FFPE produced spurious PNN-like signal. Adding perceptual loss improved fidelity but residual staining remained. VirtualPNN with anti-vessel (Percep.+AV) almost completely suppressed these artifacts. (B) Quantification of excess red signal (normalized to AI-FFPE = 1.0) showed marked reduction with Percep. (0.77) and Percep.+AV (0.24).

Conclusions & Looking Forward

Conclusions
- VirtualPNN enables accurate, label-free visualization of perineuronal nets from H&E, outperforming existing frameworks.
- Pathologist evaluation confirms high fidelity and clinical interpretability of VirtualPNN outputs.
- Novel anti-vessel loss greatly improves specificity by suppressing false-positive vascular staining.

Looking Forward
- Expand validation across larger, multi-institutional brain tumor cohorts.
- Extend framework to additional PNN markers like Wisteria floribunda agglutinin (WFA)
- Apply VirtualPNN retrospectively and correlate with brain tumor-related epilepsy prognosis

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References