Deep Learning–Based Polarization-SHG Image for Quantitative Assessment of Collagen I/II in Regenerative Porcine Knee

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INTRODUCTION: Type II collagen, which is predominantly in articular cartilage, provides tensile strength and elasticity essential for load-bearing during joint movement. However, non-destructive tools to evaluate the quality and organization of collagen within these tissues remain limited. Therefore, an accurate assessment of collagen fiber architecture is vital for evaluating cartilage health and the efficacy of regenerative ability. Second harmonic generation (SHG) microscopy provides a label-free, non-invasive imaging modality capable of visualizing fibrillar collagen. Traditional pixel-by-pixel second harmonic generation (SHG) analyses treat every pixel as an independent, discrete data point; the resulting maps often appear fragmented and computationally costly to generate. In this study, we evaluate the potential of a deep learning-based framework for polarization-SHG (P-SHG) microscopy in quantifying microscopic-scale fiber orientation and the peptide-pitch angle of the triple helix like of collagen molecule —key parameters governing collagen tissue function.

METHODS: Porcine knee samples were obtained immediately after sacrificed. Regeneration was modeled by filling full-thickness defects with an ADSC-laden fibrin hydrogel; regenerative tissues were harvested three weeks post-treatment for P-SHG imaging and subsequent neural-network analysis. To recover the true, spatially continuous architecture of collagen, we devised a physics-informed deep-learning framework that replaces isolated pixel fitting. The model is pre-trained on large-scale simulated P-SHG stacks created with forward theoretical models, then refined by transfer learning on a small set of experimental P-SHG images from porcine knee cartilage. In the first stage in Fig. 1(A), pixel-masked MNIST-style images are arranged in random distribution patterns to introduce spatially coherent collagen features. In the second stage (Fig. 1B), tissue samples are imaged with a dual-liquid-crystal P-SHG system, producing 256×256-pixel stacks with 18 polarization states. During inference in Fig. 1(C), a new tissue stack acquired by the same system is passed to the loaded deep-learning model, which outputs high-resolution maps of fiber orientation and peptide-pitch angle within milliseconds.

RESULTS: We evaluated the performance of our deep learning framework using well-known tendon tissue samples, which serve as a well-defined reference due to their highly aligned collagen structure. As shown in Fig 2, we compared the results of three approaches: (i) pixel-wise theoretical fitting, (ii) a pre-trained physics-driven model, and (iii) our proposed transfer-learned physics-driven model. This approach enables realistic, spatially coherent collagen maps in ~200 ms per sample, boosting image smoothness by 78 % and cutting total variation by 32 % compared with traditional pixel-wise fitting. As shown in Fig. 3, we applied the transfer-learned physics-driven model to analyze collagen architecture in subchondral bone, cartilage, and regenerative tissue obtained from porcine knee samples. It accurately distinguishes and quantifies type I and type II collagen in regenerated cartilage and underscoring the advantage of a neural-network approach over discrete pixel-based methods.

DISCUSSION: The physics-informed deep-learning framework, which combines simulation-based pre-training with transfer-learning fine-tuning on experimental data, delivers fast, label-free, and non-destructive collagen quantification. These results confirm both the feasibility and the effectiveness of the approach, underscoring its strong potential as a research and diagnostic tool for monitoring cartilage repair.

SIGNIFICANCE/CLINICAL RELEVANCE: This work highlights the potential of integrating P-SHG imaging with deep learning for clinical translation, offering new opportunities for personalized diagnostics, early detection of tissue degeneration, and evaluation of regenerative treatments.

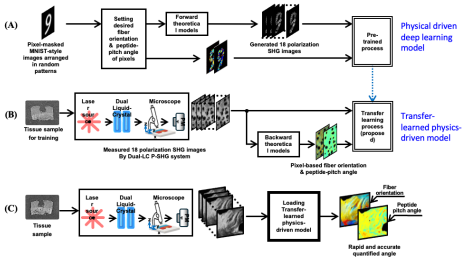

Fig.1 Physics-driven P-SHG deep-learning workflow for collagen quantification. (A) The synthetic stacks (140,000 in total) are used to pre-train a physically driven deep-learning model. (B) Transfer learning with experimental data from porcine knee samples were used to adapt the model to real-world imaging conditions. (C) The adapted model delivers.

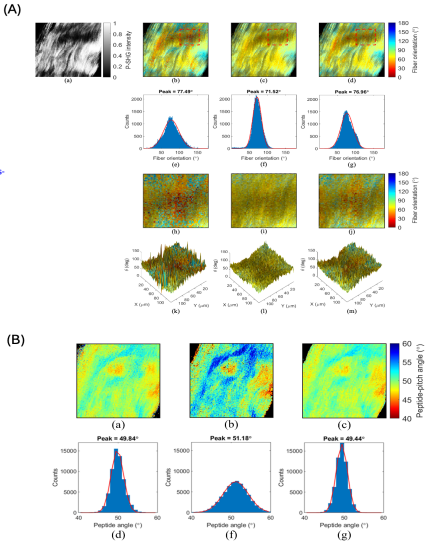

Fig. 2. (A) Results of collagen fiber orientation in tendon samples using different analysis approaches. (B) Results of peptide pitch angles in tendon samples using different analysis methods.

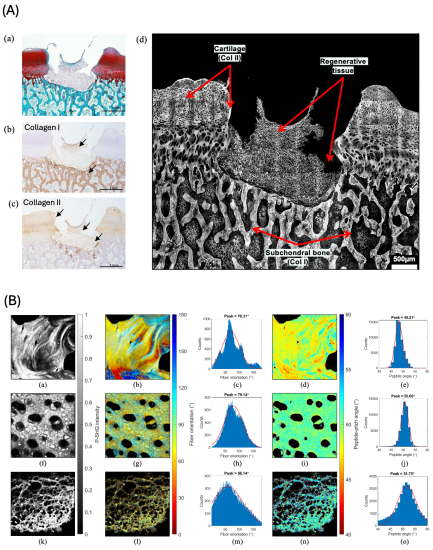

Fig. 3. (A) Data preparation and qualitative comparison between histological staining and SHG imaging of tissue samples. (B) Results of subchondral bone, cartilage, and regenerative tissue using the transfer-learned physics-driven model.