A computer vision framework for quantifying histological differences in intervertebral discs

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INTRODUCTION: Intervertebral disc (IVD) degeneration in the lumbar spine is frequently associated with low back pain, the world's leading cause of disability. Because the degeneration process is poorly understood, preclinical animal models are widely used to investigate IVD degeneration and regeneration. The rat lumbar spine is a common preclinical model for studying IVD due to ease of handling, relatively large size, and anatomic and mechanical similarity to humans[1,2]. Tissue features are often evaluated through histopathological staining and imaging to visualize IVD cellular characteristics and anatomic morphology and assess the state of degeneration, often through various semi-quantitative grading systems. Recent efforts have harmonized these grading systems, however there is no fully quantitative system that can replace the inherent bias among human graders. Computational digital pathology has the potential to limit human involvement in histological grading and eliminate the variability between graders. For this study, we developed a computational pipeline that incorporated deep learning and computer vision to numerically abstract IVD histopathology images. We hypothesized that the computational pipeline could detect underlying visual features that define the nucleus pulposus (NP) and annulus fibrosus (AF).

METHODS: This study was approved by the Duke University animal care committee. *Histological analysis* Twenty-four rats from 3 age groups were evaluated histologically: 10 weeks (4 male, 4 female), 6-9 months (4 male, 4 female), 20 months (4 male, 4 female). Levels L5-L6 and L6-S1 were harvested from the rats, decalcified, fixed in formalin, and embedded in paraffin wax. The embedded samples were sectioned on a microtome at 8 µm, stained with Harris Hematoxylin and Eosin Y (H&E), and imaged at 400x (Leica Aperio AT2), resulting in 48 IVD whole slide images (WSI). *Computational modeling* WSIs were digitally processed by applying stain normalization (Macenko's Method), background removal, and patching (300 by 300 pixels by image patches). This resulted in 102,340 image patches from 48 WSIs of the IVDs. A computational deep learning pipeline was developed to embed the image patches in latent feature space. The pipeline was developed on top of a pre-trained deep convolutional neural network (CNN) specialized for computer vision. We used the AlexNet architecture pretrained on ImageNet and repurposed the network via transfer learning with a public dataset of 100,000 image patches of colorectal cancer pre-labeled at the patch level with 9 histological categories. We used a 80% training, 10% validation, and 10% test data split and achieved a 99.7% training accuracy and 99.1% validation accuracy on the colorectal cancer dataset. The trained model was used to process the IVD image patches for feature extraction. We defined the feature vector as the final 1024x1 fully connected layer before classification, extracting 1,024 later features from each patch. Principal component analysis (PCA) was performed to reduce the size of the data (102,340 by 1,024) to 100 principal components (95% cumulative variance) and uniform manifold approximation and projection (UMAP) was utilized for 2D visualization. To group visually similar images, K-means clustering was used to cluster the PCA-reduced feature vectors and silhouette score was used to determine optimum cluster number. All image analysis was performed on a computational workstation (HP Z2, processor: i9, RAM: 128 GB, GPU: NVIDIA A2000).

RESULTS: We identified mild degeneration in the rat intervertebral disc with age, where 20-month rats had evidence of fibrosis in the inner AF and NP (Fig. 1A). Feature extraction and clustering analysis revealed 9 clusters, suggesting 9 visually similar species of image patch. Early results suggest that we can grossly differentiate the AF (cluster 0) and NP (cluster 1) (Fig. 1B). UMAP embedding demonstrated evenly distributed patch clusters, with one long tail (Fig. 1C, lower right) that included background patches.

DISCUSSION: Preclinical models are vital to IVD research to assess and investigate the mechanisms driving disc degeneration and regeneration. The application of computational digital pathology methods such as CNNs to histopathology can aid researchers by improving the precision and accuracy of histological analysis. better accuracy in studying IVD degeneration by reducing human error and variability on histological grading on IVD morphology. We hypothesized that we would find certain patterns related to IVD morphology. Early results suggest that we can grossly differentiate major regions of the disc (NP, AF) and background artifacts. Future work will need to refine these methods to isolate growth plate, bone, and cartilage endplate. In conclusion, we developed a promising computational tool to cluster histological image patches based on relevant IVD morphology. Future work will refine the tool, develop numerical assessment of pathological features (e.g., pathological area), and explore applications in other tissues.

SIGNIFICANCE: We developed a computational tool to classify IVD morphology through clustering and to further investigate underlying patterns related to IVD degeneration. This tool could be used in future work to quantify visual differences between any musculoskeletal tissues.

REFERENCES: 1) O'Connell+ Spine 2007; 2) Beckstein Spine 2008;

Figure 1 – (A) Representative images from rats at each time point. 20 month rats appeared to be mildly degenerated, particularly in the inner AF region. (B) Representative images of AF and NP clusters grouped via feature extraction and cluster analysis. (C) UMAP embedding demonstrating distribution of 9 clusters of image patches with visual similarity.