Nano-CT Image Processing and Micromechanical Modeling of Intermuscular Bone in Herring

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Introduction

Intermuscular bone is a type of tissue exclusive to teleosts, such as North Atlantic Herring. It is formed by the intramembranous ossification of myoseptal tendons, making it less cartilaginous. The goal of this study is to use Nano-CT imaging on herring previously subjected to mechanical tensile testing to further investigate the microstructure of intermuscular bone and analyze the changes in mechanical properties and porosity related to aging. Teleost intermuscular bones exhibit unique mechanical properties due to the relationship between their structure and function. This study has been using 30nm and 100nm advanced high resolution scans of intermuscular bones in herring of two cohorts with a sample size of 2 for both the small fish and the large fish. The aims of this have been to identify and quantify differences in density and porosity as well as other structural features in the intermuscular bone and to start developing a micromechanical code to analyze their mechanical properties and relate them to the microstructural features.

Methods

Image Acquisition and Processing

In this study, nano-CT scans of herring bone at the 100nm and 30nm level were done and the output of the scans was in the form of .VOL files. This is a compressed volume file extension that stored 33GB of data for each scan. The first images were acquired and analyzed by importing the .VOL files into the image processing software ImageJ. Then, the files opened in ImageJ were exported as a stack of 2048 slices across the bone. Using ImageJ the x,y,z and pixel values at different regions on each slice were identified. The pixel values represent the density value at a specific region. Bone has the maximum density value, while pores and air have the minimum density values. The values were mapped using a formula relating attenuation to bone density and were used to convert the TIFFs to DICOMs. The DICOMs were imported into MIMICS and were processed using morphological operations and segmentation techniques to separate the pores from the bone and create a 3D model of the pores.

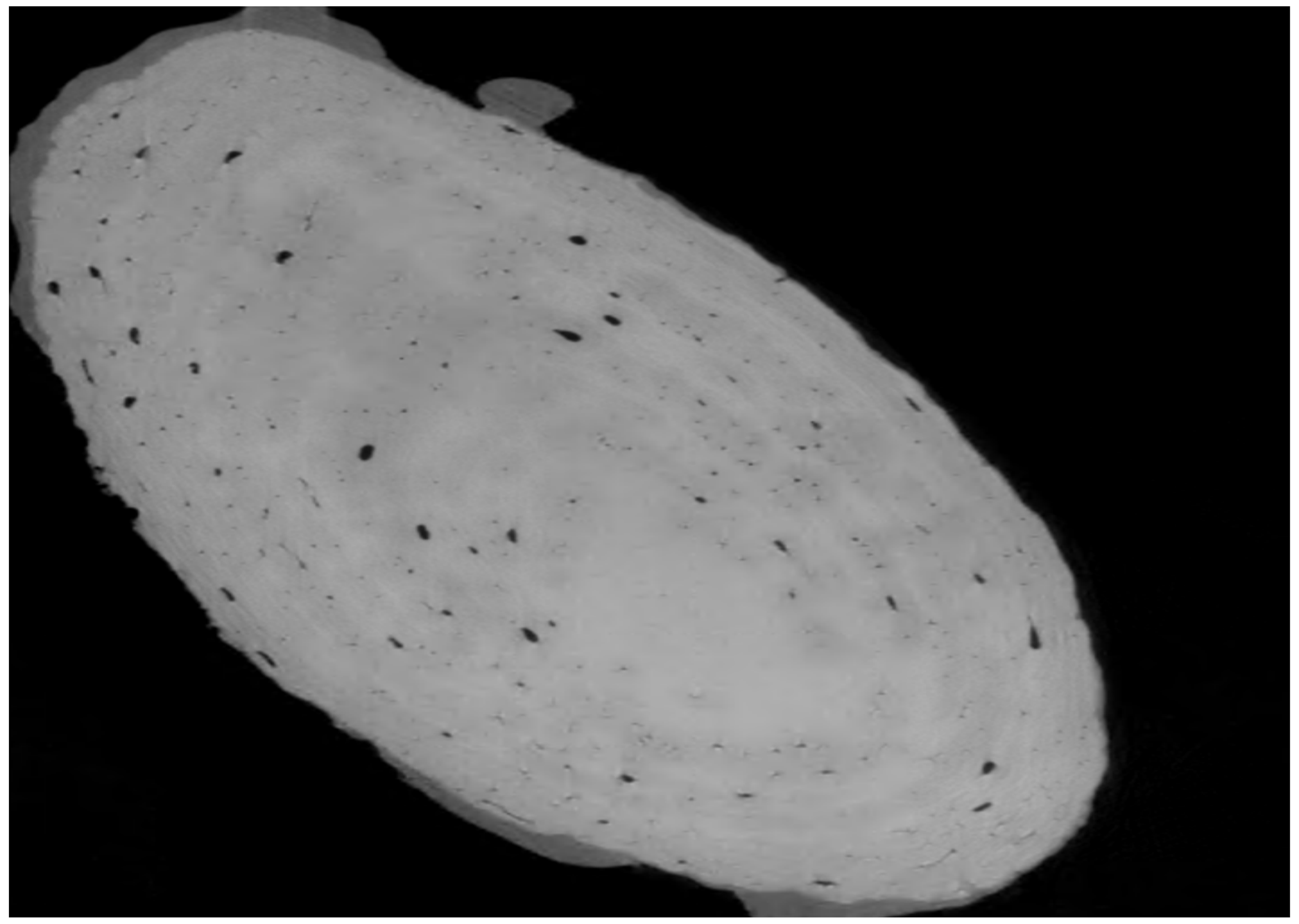

Figure 1: First image acquired of the nano-CT bone scans. The white color indicates bone and the black color indicates pores.

Results

The result of the image processing was multiple three dimensional models generated for a significantly large set of digital images. This enabled the visualization of the intermuscular bone structure and the analysis of the pores and the patterns created by the pores in the different bones. Each network of pores is unique and can be analyzed to show how it affects the material properties of the bone. The images generated are at a scale that has not been seen before and show the specific pores left by the cells, and not just the structural pores, at an incredibly detailed level.

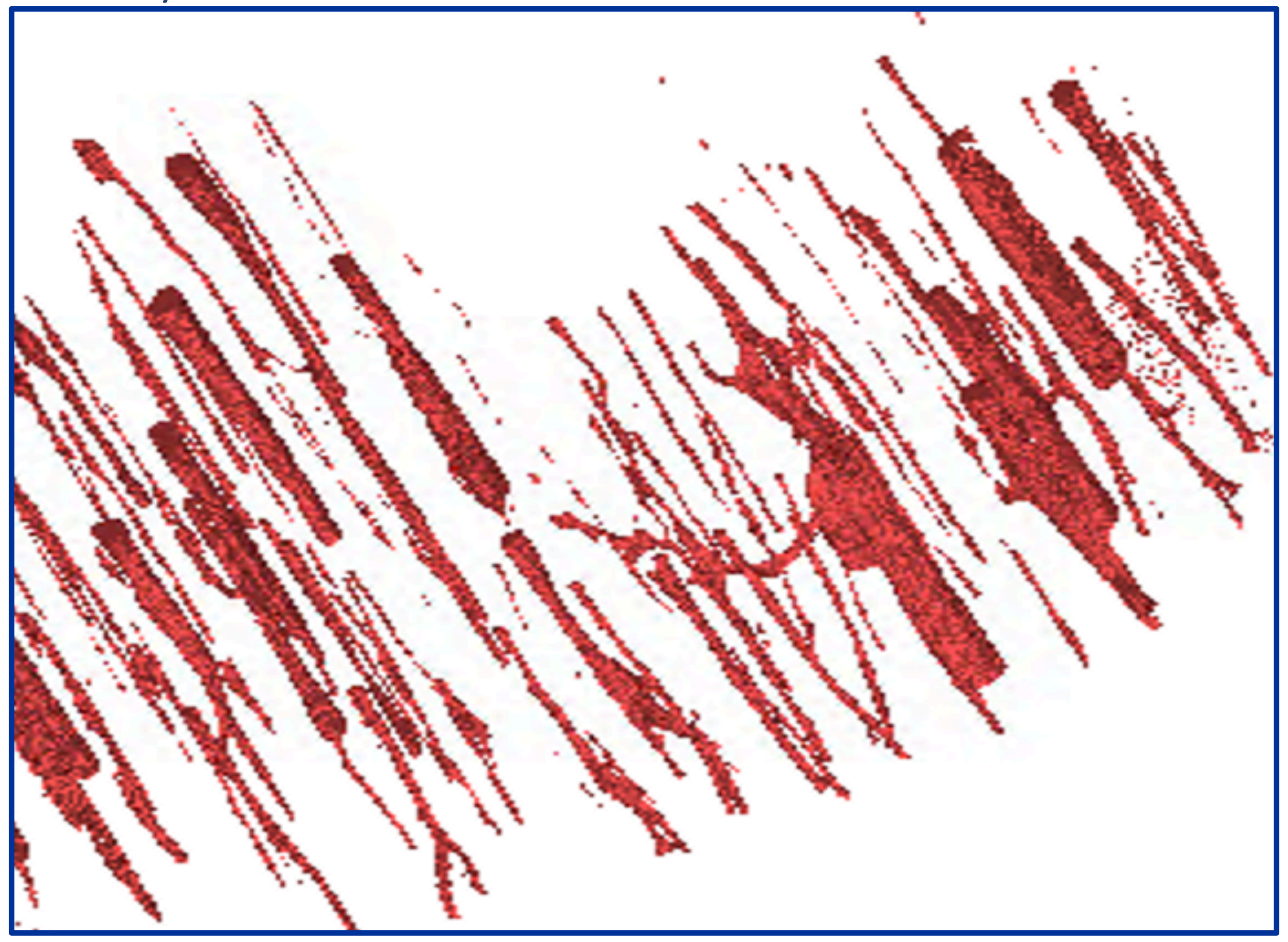

Figure 2: A cross-sectional view of the 3D model generated of the pores within the bone.

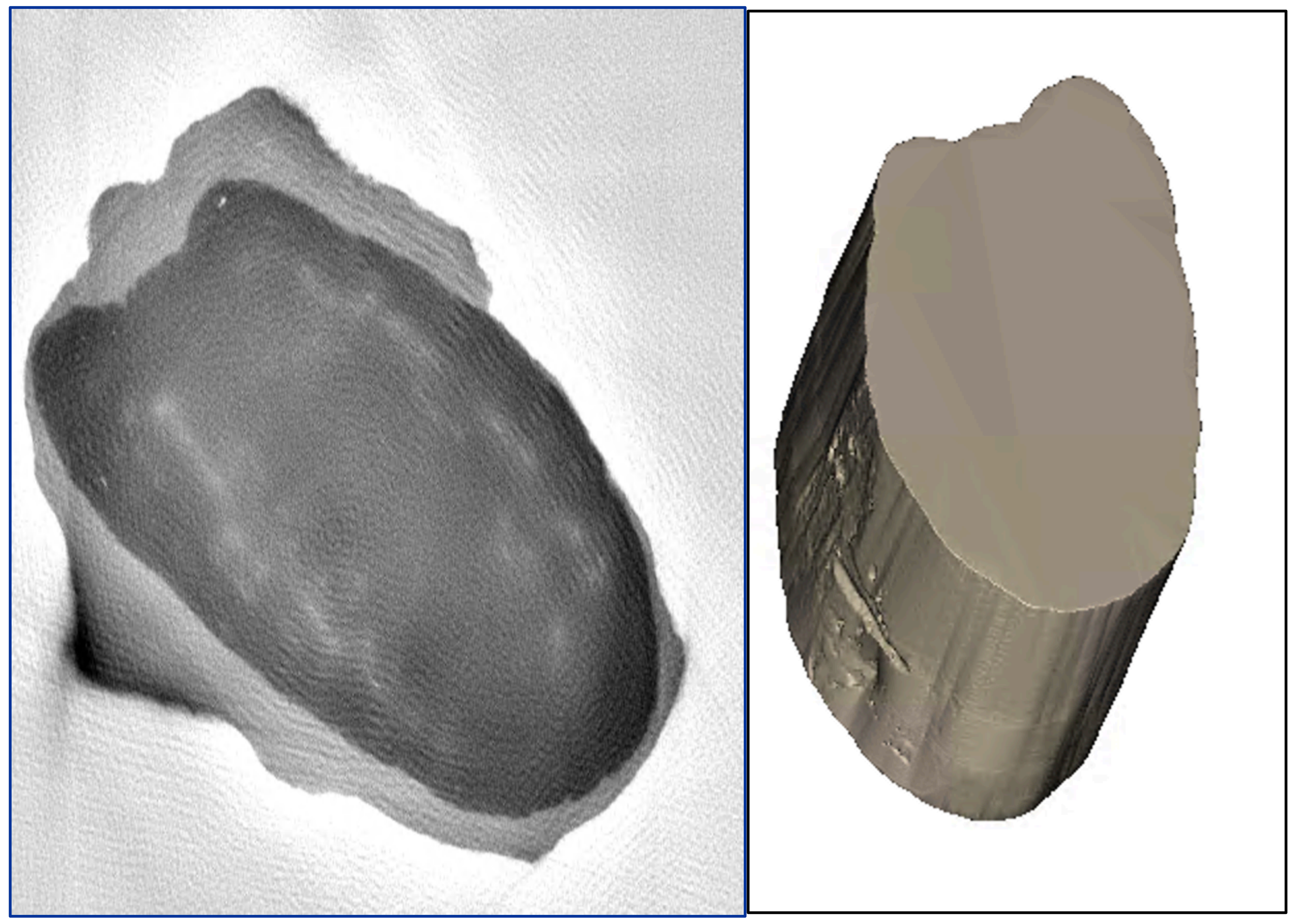

Figure 3: A 3D model generated of the intermuscular bone of a small fish.

Discussion

Application of Generated Results

The models generated by this study are significant as they can help in looking at the microstructure of bone. At the bulk level, which is the centimeter scale, the elastic modulus is related to density but at the tissue level, which is the micrometer to millimeter scale, the relationship between elastic modulus and density is less significant. On the other hand, structural components in the microstructure of the bone, such as porosity and mineral crystal organization, significantly impact the mechanical properties of the bone (1). Additionally, diseases like chronic kidney disease (CKD) impact the microstructure and material properties of bone resulting in reduced bone quality (2). Therefore, the techniques in this study could be applied by trying the newly established nano-CT image processing framework on CKD and other bone diseases that cause micro-porosities.

Current Data and Future Studies

homogenization will be applied using the representative volume element identified for each intermuscular bone scan and that will be the repeating unit that contains enough microstructural features to be representative of the entire bone as a composite. BoneJ can be used to crop the repeating element that will be used in the micromechanical code. This study on the intermuscular bone samples will use the micromechanical coding model in Lau et al.'s study . The code runs using the software MAC/GMC and the concept of homogenization and generalized method of cells. The development of the micromechanical code for this study will include a third element, which is pores and accounts for intermuscular bone as a three part composite. Running the MATLAB code creates a MAC/GMC output file.

Conclusions

The scanned images and .VOL files were opened and used to generate TIFFs and DICOMs. An image of the bone was visualized using ImageJ. A relationship was established between density and attenuation based on the raw data, and the large data sets of pixel values in ImageJ were converted to attenuation values and imported into MIMICS. A 3D model of the image stacks was created and processed to visualize the appearance of the pores and microstructure of bone. Overall, this study established the image processing work flow for nano-CT scans.

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