Geometric Models and Image Analysis Algorithms for Detecting Skeletal Phenotypes in Zebrafish

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Introduction

Our goal in the MSBL is to identify the causal genes for osteoporosis and osteoporosis genetic risk factors with high throughput phenotyping using a zebrafish model. Osteoporosis is characterized bone expansion due to accelerated bone remodeling. Our current technology for phenotyping using microCT images can take measurements of thickness, length, and mineral density for the neural arch, haemal arch, and centrum of each zebrafish vertebra.[1] It cannot however quantify expansion of these structures, which is the aim of my research project. We approached this problem by developing geometric predictive models and direct quantification image analysis programs.

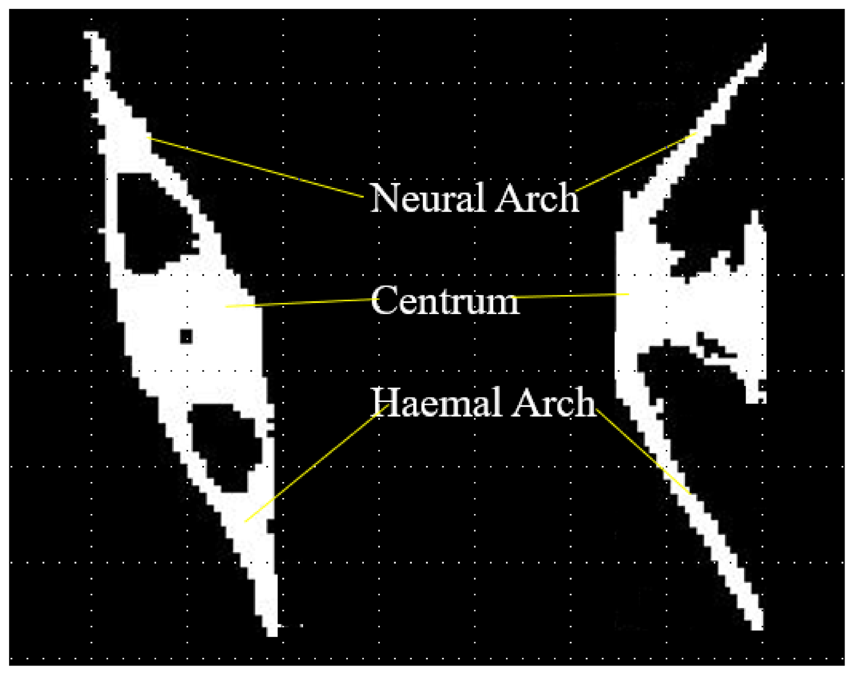

Fig 1: Diagram of zebrafish vertebral structures.

Geometric Models

- Used integral calculus to develop two models that depend on measured length, thickness, and volume of each centrum
- Due to variable centrum shape, we combined the models to predict based on centrum location
- Model Equation: D is diameter, S is a multiplier that determines concavity, L is length, and T is thickness

$$D = S\left(\frac{V}{\pi \cdot L \cdot T} - T\right)$$

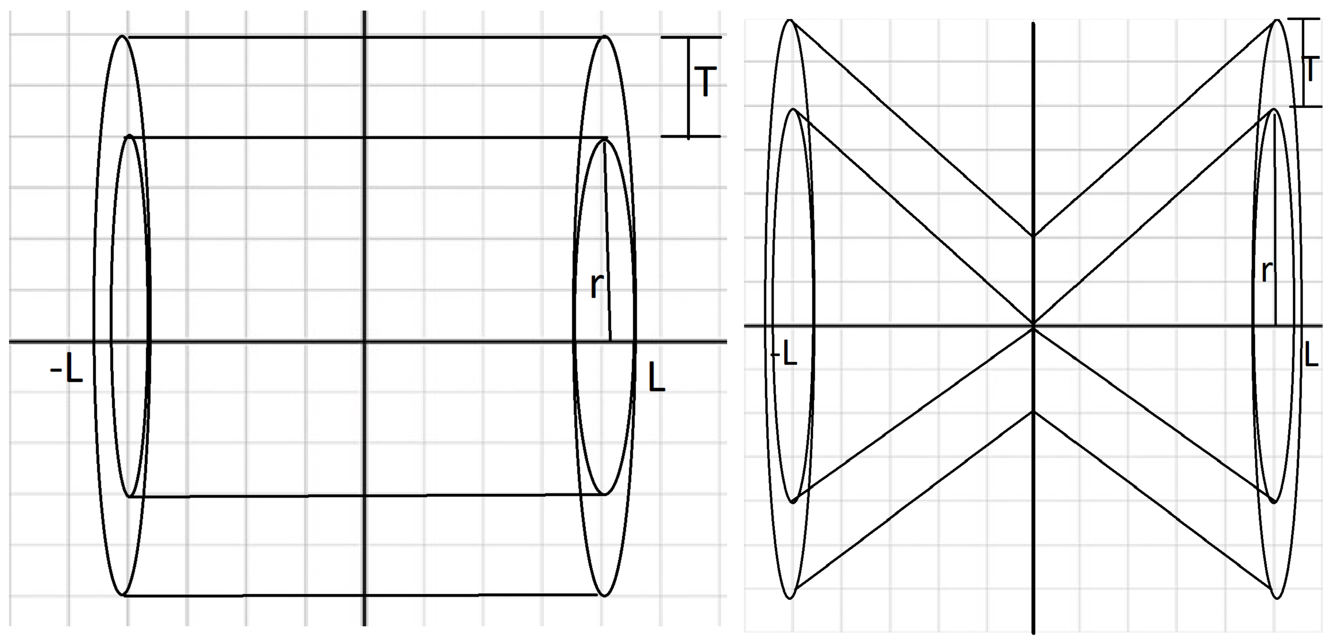

Fig 2: Mathematical and geometric representations of centrum model.

Limitations

- Works well for precaudal vertebrae, but too much variation in caudal vertebrae to be generalizable
- Length measurement depends on human input, and is susceptible to inaccuracy due to human error

Direct Quantification

- Expand on existing image analysis program
- Use previously collected data to ensure compatibility
- Measure foramen of neural arch, haemal arch, and centrum

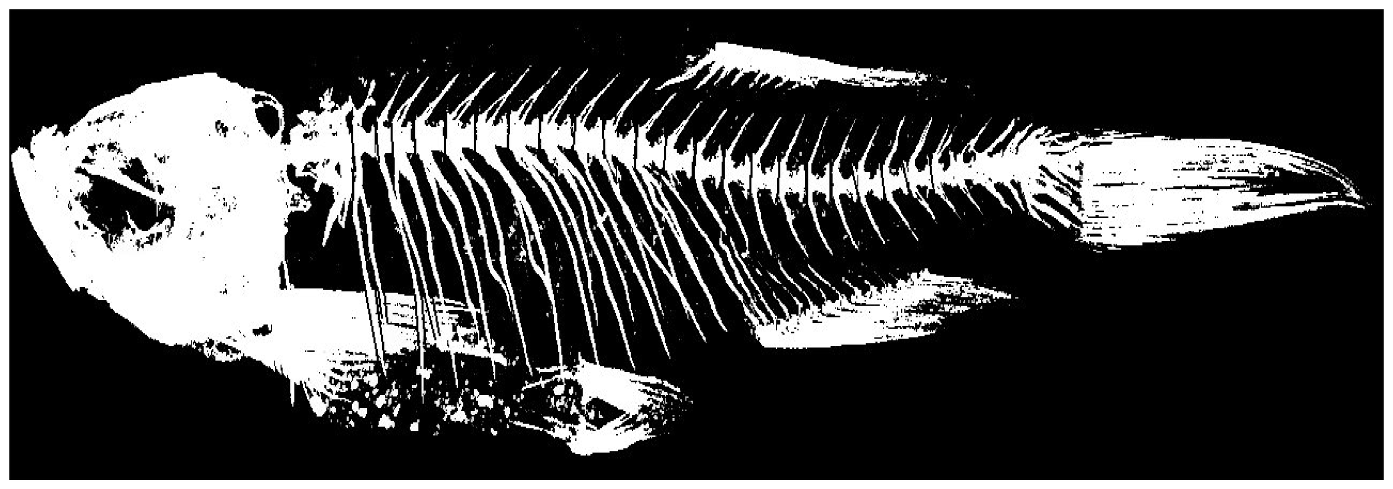

Fig 3: Projection of manually segmented binarized microCT stack of zebrafish skeleton: input file for processing

Centrum Measurement Prototype

- Uses vertebral boundary coordinates from user input to identify starting slice
- Slices from posterior to anterior until a slice with a fully closed centrum is detected
- Measures the cross-sectional area of the centrum foramen

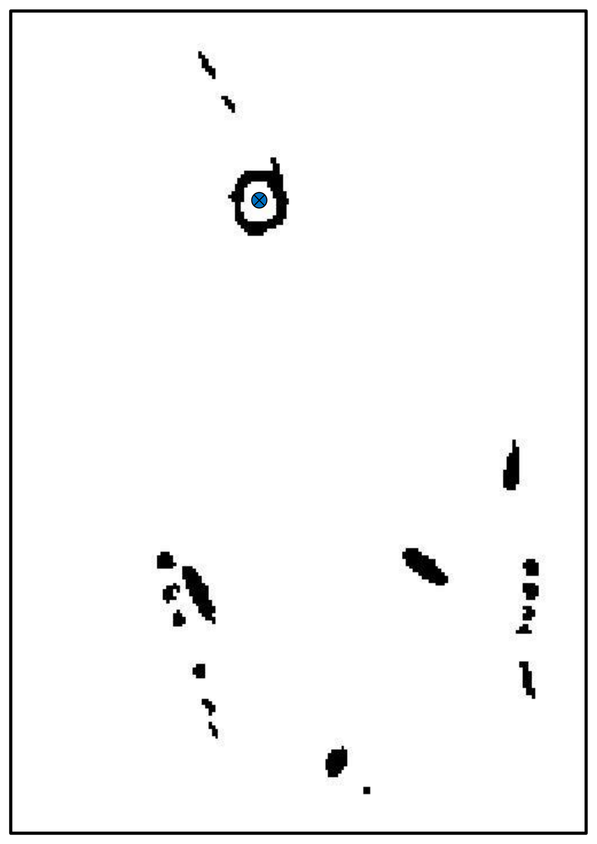

Fig 4: Processed image of detected centrum foramen.

Arches Measurement Prototype

- Uses vertebral boundary coordinates from user input to identify projection range
- Creates a max intensity projection axially through the vertebra
- Measures the area of the neural and haemal arches in the max intensity projection

Fig 5: Processed images of detected neural arch foramen (left) and haemal arch foramen (right).

Advanced Arch Measurement

- Accounts for the angle of the arch (only currently implemented in the neural arch)
- Isolates the arch in a maximum intensity projection of the vertebra
- Calculates the major axis orientation angle and rotate to make the neural arch vertical
- Computes the bounding box for the neural arch to use as projection range
- Rotates entire microCT stack of the vertebra
- Takes maximum intensity projection through the rotated stack with the bounding box range
- Measures major and minor axes of the arch foramen

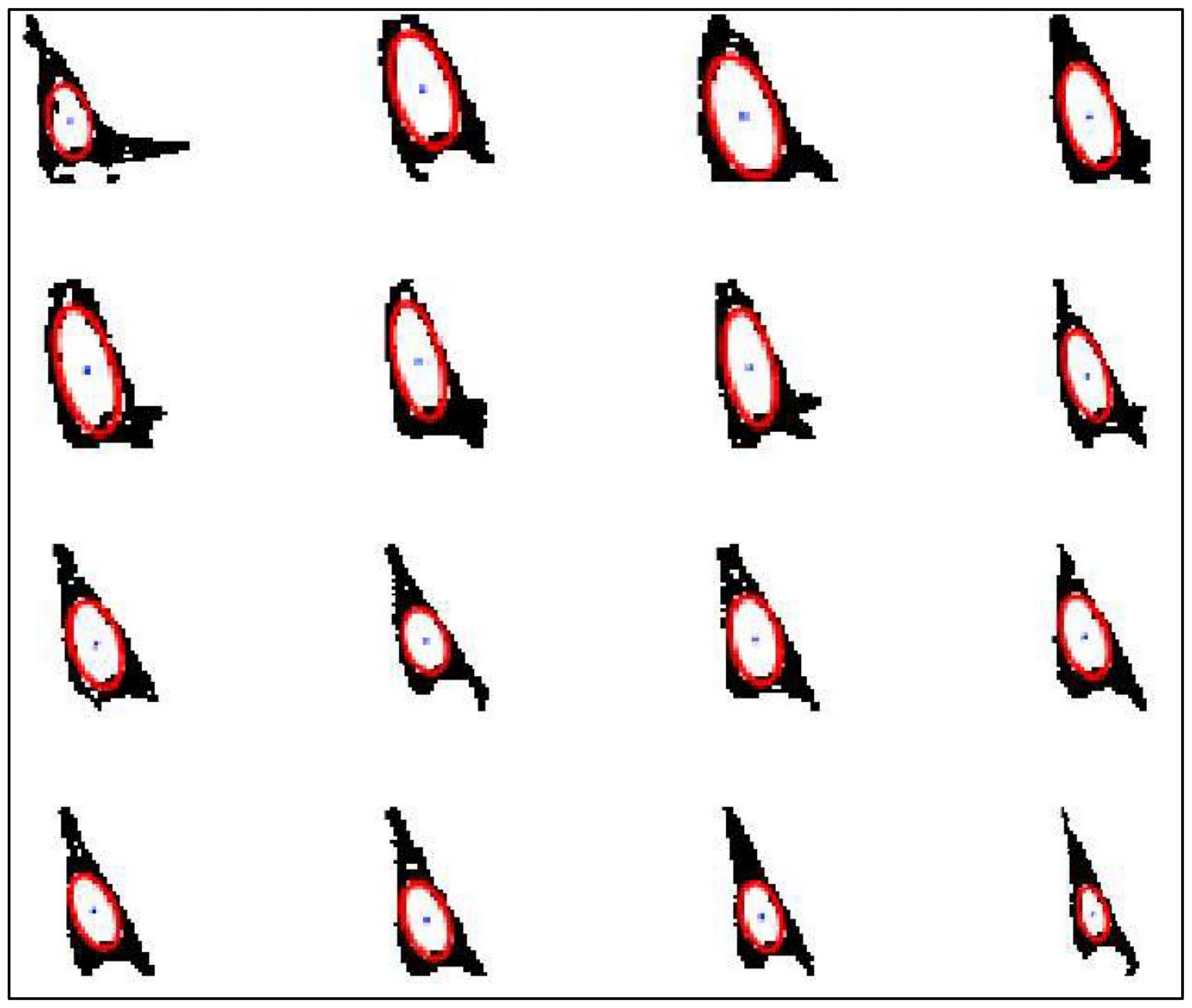

Fig 6: Processed images of detected and measured neural arch foramen using stack rotation algorithm.

Discussion

- Centrum measurement prototype is inaccurate when measuring fused vertebrae or heavily compressed vertebrae
- Advanced arch measurement program is more accurate because it accounts for arch angle, but it is less consistent than the prototype
- We developed a geometric model and image analysis technologies to increase our lab's skeletal phenotype detection capabilities

Acknowledgements

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References

1. Hur M, Gistelinck CA, Huber P, et al. MicroCT-Based Phenomics in the Zebrafish Skeleton Reveals Virtues of Deep Phenotyping in a Distributed Organ System. Zebrafish. 2017.