FibroQuant: ImageJ-Based Color Thresholding to Differentiate Amyloid Transthyretin from Healthy Controls via Fibrosis Signal Analysis

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Abstract

FibroQuant is an ImageJ-based color thresholding method developed to quantify fibrosis in heart tissue aiming to differentiate amyloid transthyretin (ATTR) from healthy controls (HC). The process involves setting custom color thresholds to capture fibrosis signal areas in microscope images. The percent fibrosis signal is calculated by dividing the fibrosis area by the total heart tissue area. In this blinded study we observed that female ATTR mice showed significantly higher fibrosis levels compared to controls (p<0.01), while male mice did not show significant differences. Although no significant gender differences were observed in fibrosis levels, ATTR mice overall displayed higher fibrosis compared to controls. These results confirm that FibroQuant can successfully differentiate between ATTR and HC samples offering a reliable method for fibrosis quantification in cardiomyopathy research.

Introduction

- **Amyloid Transthyretin-Related Cardiomyopathy (ATTR-CM) -** A condition in which amyloid proteins misfold and accumulate in the heart leading to stiffness and restrictive cardiomyopathy, which can cause heart failure and potentially death.
- Our ImageJ-based color thresholding method aims to provide a streamlined and consistent way to quantify the amount of fibrosis within a sample heart tissue.
- Goal: Use FibroQuant to differentiate heart tissue samples as amyloid transthyretin (ATTR) or healthy control (HC) based on the percent of fibrosis signal area relative to overall heart tissue.
- **Hypothesis: FibroQuant will be able to differentiate between amyloid transthyretin (ATTR) and healthy control (HC) mice samples using fibrosis signal analysis.**

Methods

The experiment is blinded with the data analyst unaware of what is ATTR vs control samples.

Procedures:

1. Import the 10x or 20x microscope zoomed sample picture into ImageJ/Fiji.
2. Use Image -> Adjust -> Color Thresholds to set custom color thresholds for the image.
3. Set the hue to 171-187, saturation to 0-255 and brightness to 0-225 to capture the blue signal area which would be fibrosis in the heart sample.
4. Select the signal area and measure the amount of fibrosis within the sample.
5. Again, Set the hue to 151-189, saturation to 0-255 and brightness to 0-225 to capture the red and blue signal area which would be the total area of fibrosis and healthy heart tissue combined.
6. Divide the total area of the blue signal area by the red and blue signal area to get the percent fibrosis of the sample.

Methods

Example:

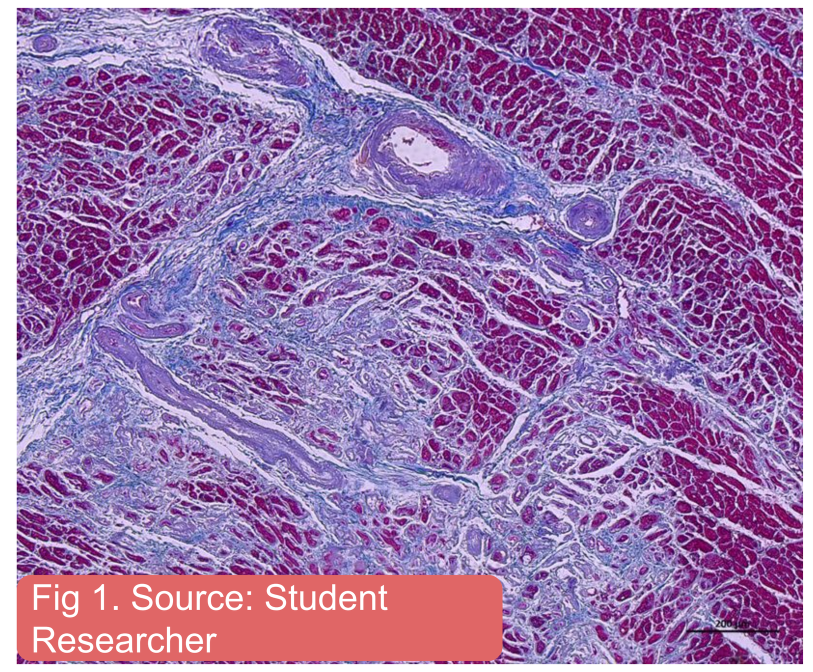


Fig 1. Source: Student Researcher

Amyloid Patient (AL) Left Ventricle

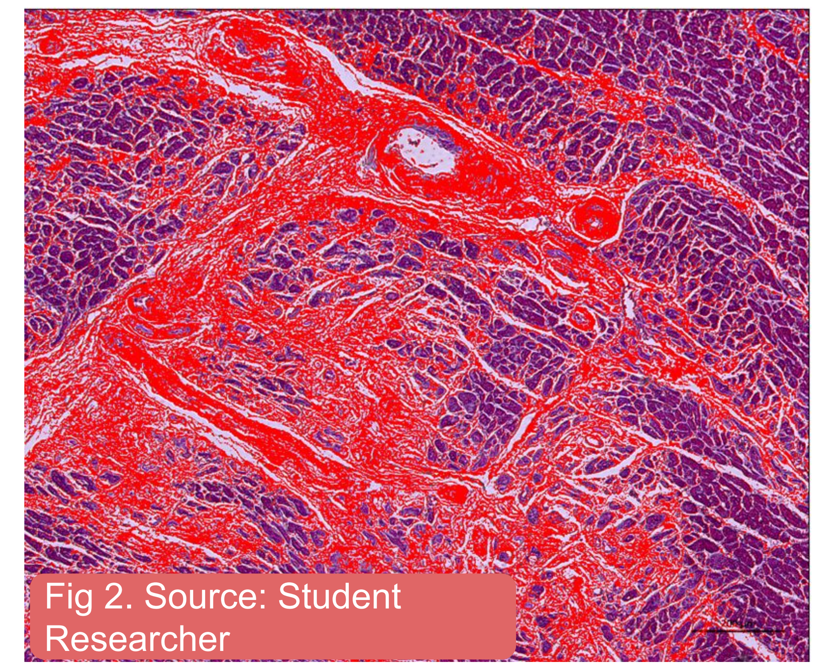


Fig 2. Source: Student Researcher

Threshold Color Hue: 171-187, Full Saturation Brightness: 0-225

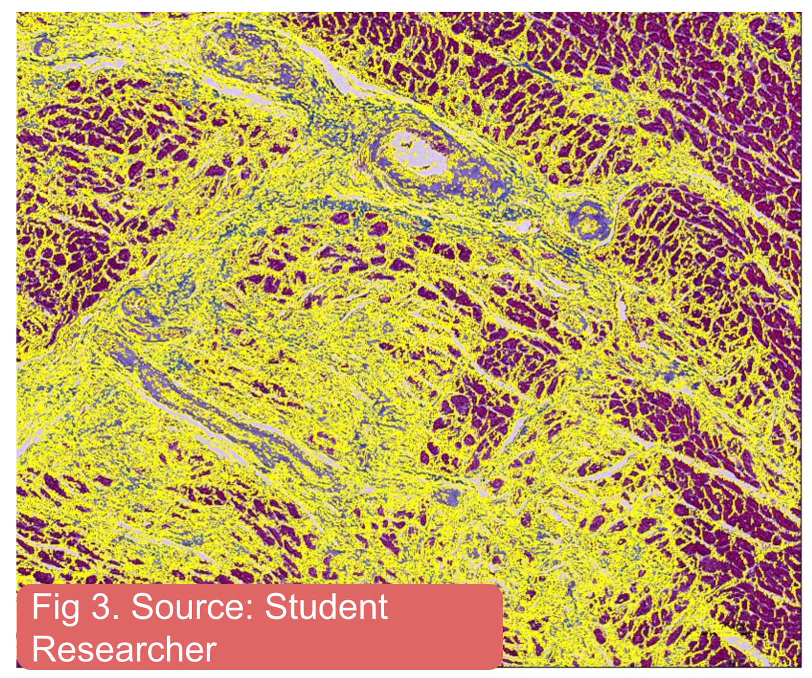


Fig 3. Source: Student Researcher

Select & Measure Pixel area

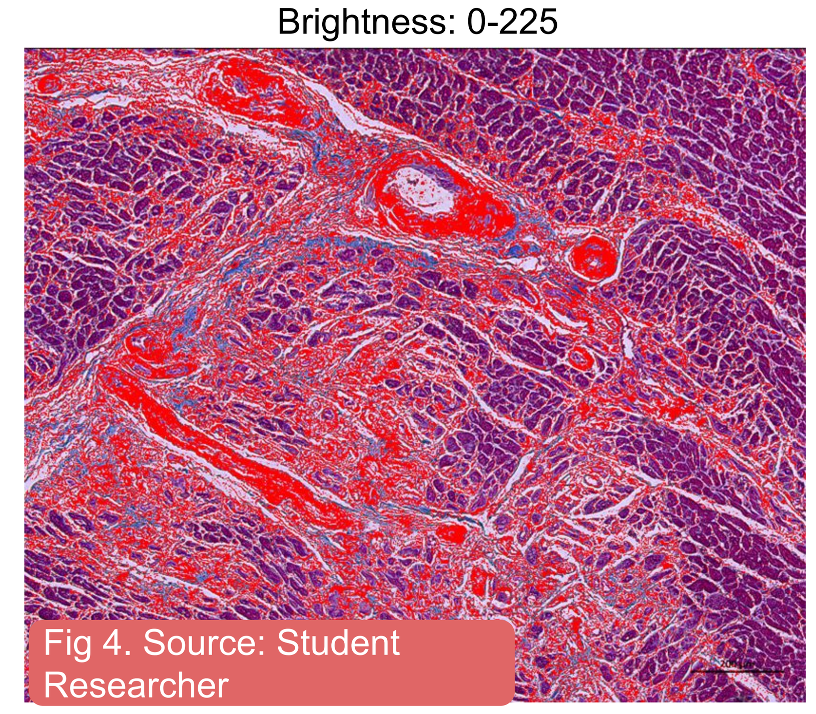


Fig 4. Source: Student Researcher

Threshold Color Hue: 151-189, Full Saturation Brightness: 0-225

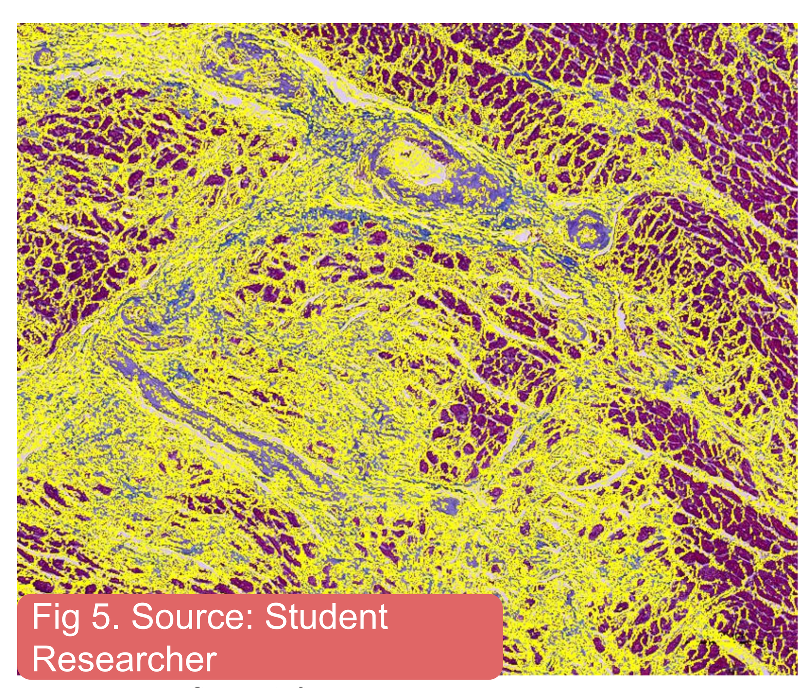


Fig 5. Source: Student Researcher

Select & measure pixel area

Blue (1,333,787) + Red = 1,954,097 pixels

$$\frac{Blue\ Channel\ Area}{(Blue+Red\ Channel\ Area)} * 100 = \%\ Fibrosis$$

$$\frac{1,333,787}{1,954,097} * 100 = 68.3$$

- Completed 300 mice samples
- 100 Female Mice Samples
- 200 Male Mice Samples
- 10x & 20x microscope images

Outcomes

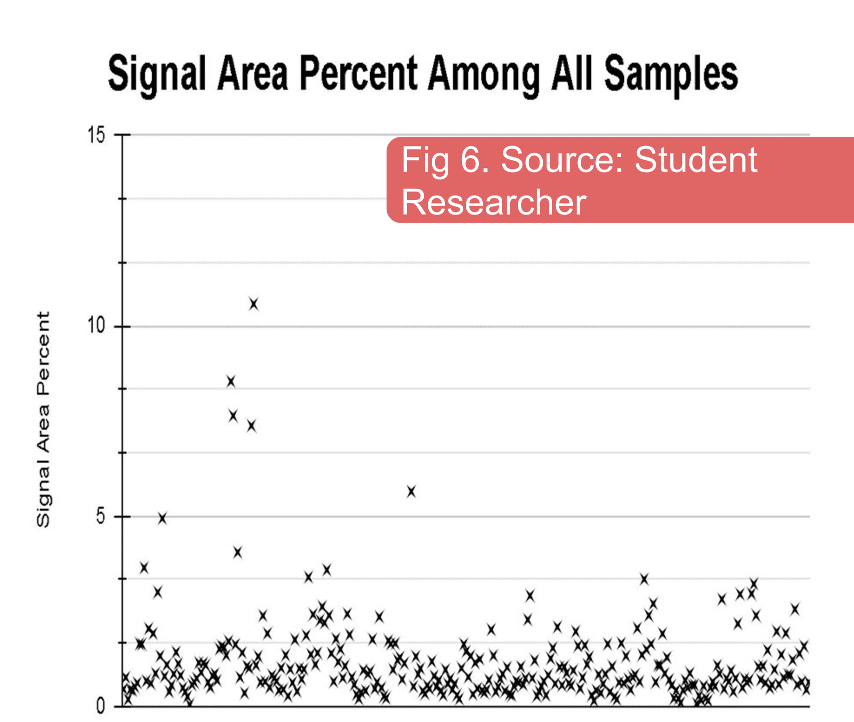


Fig 6. Source: Student Researcher

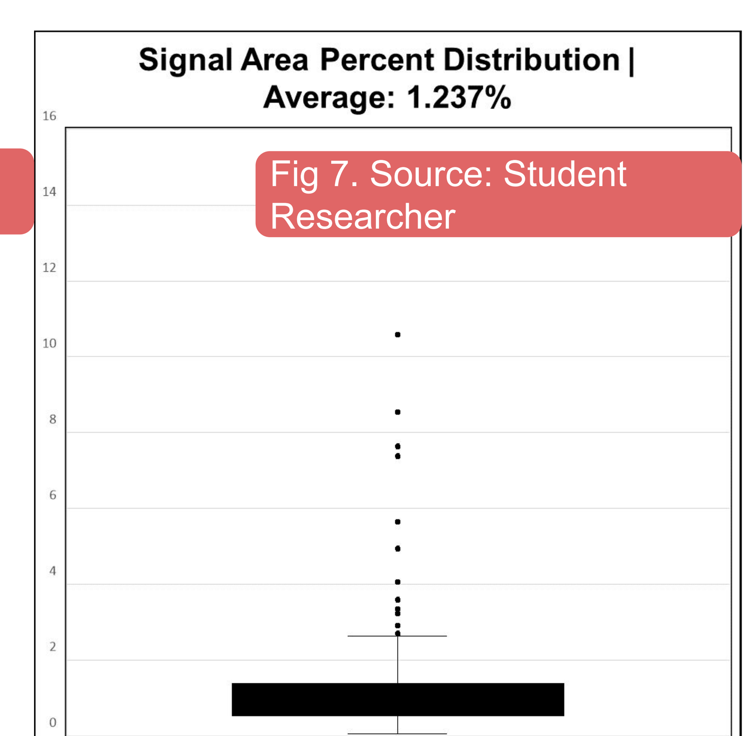
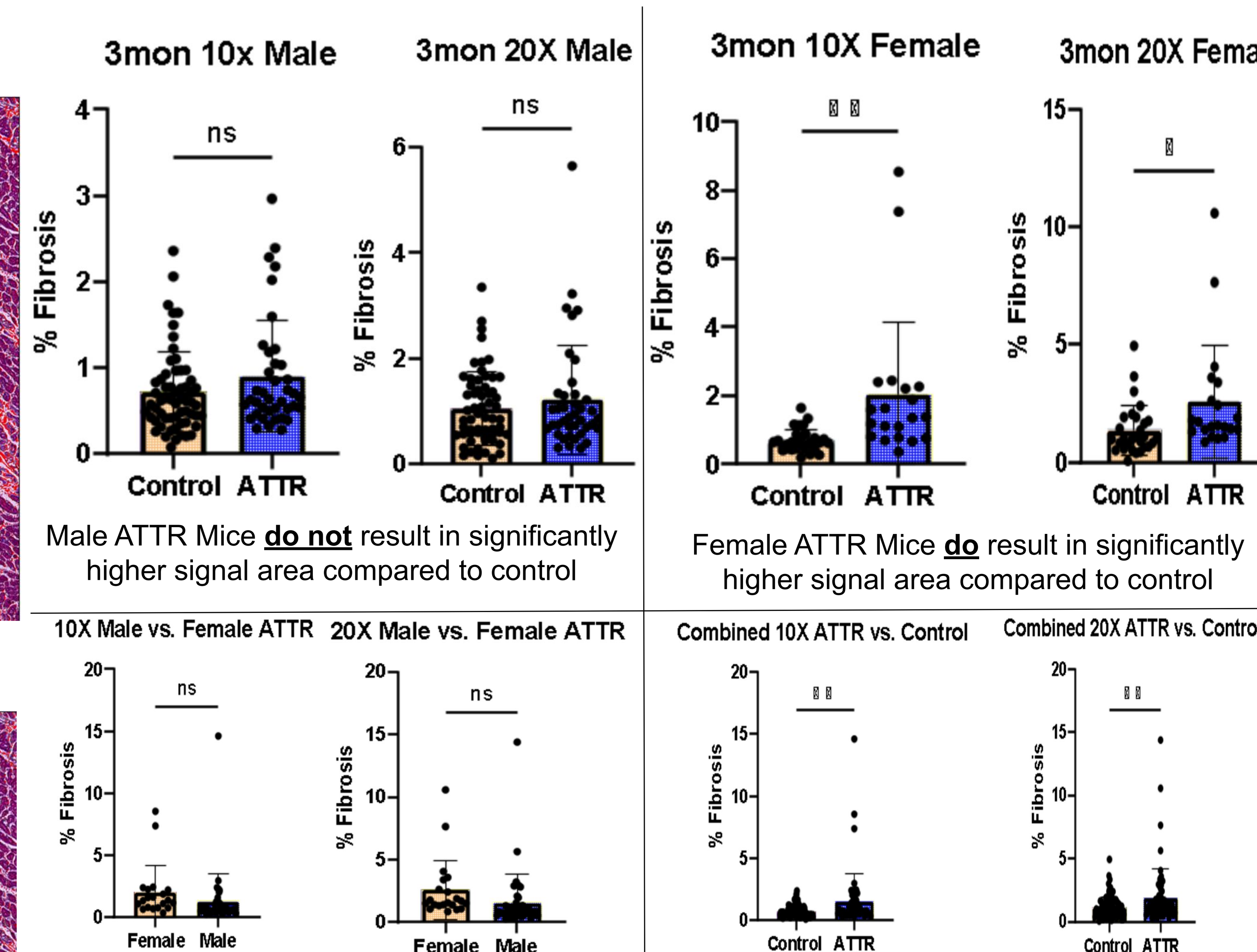


Fig 7. Source: Student Researcher

Outcomes



Male ATTR Mice **do not** result in significantly higher signal area compared to control

Female ATTR Mice **do** result in significantly higher signal area compared to control

Differences in gender **do not** result in significantly higher signal area

ATTR Mice **do** result in significantly higher signal area compared to control

Fig 8. Source: Student Researcher

Analysis

- 3 month 10x male: Control vs ATTR - p>.05 (not significant)
- 3 month 20x male: Control vs ATTR - p>.05 (not significant)
- 3 month 10x female: Control vs ATTR - p<.01
- 3 month 20x female: Control vs ATTR - p<.05
- 10x Male vs Female ATTR: Female vs Male - p>.05 (not significant)
- 20x Male vs Female ATTR: Female vs Male - p>.05 (not significant)
- Combined 10x ATTR vs Control: Control vs ATTR - p<.01
- Combined 20x ATTR vs Control: Control vs ATTR - p<.01

Conclusions

The hypothesis was correct that FibroQuant will be able to differentiate between amyloid transthyretin (ATTR) and healthy control samples (HC) based off the fibrosis signal area. The male mice did not show a statistically significant difference between HC and ATTR mice while the female mice did. Although there was no significant difference between female and male fibrosis, **overall ATTR mice show significantly higher levels of fibrosis compared to HC mice, allowing us to differentiate between amyloid transthyretin (ATTR) and healthy control (HC).**

References

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