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Stanford University Alexander Lab Research Internship Journal

Resources

[Alexander Lab Website](#)

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Summer 2024

Week 1: 06/17/2024

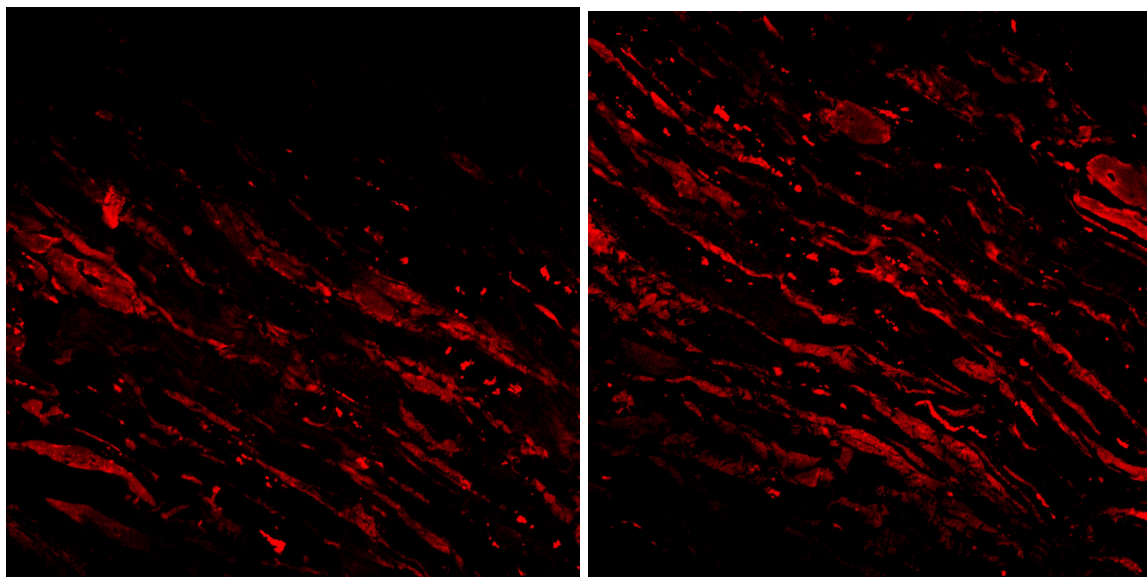
Today is the first day of the internship.

Goals by the end of the internship:

- Write and publish a literature review.
- Write a full report summary on an experiment done with data from research.
- Convert the BUMP research poster into a research paper and publish it onto Arxiv or a journal.
- Possibly get additional data on the BUMP device at the lab and tweak the poster.

My role in the internship will be predominantly analyzing data and images. I plan on using various statistical software such as REDCap, Prism, Stata and ImageJ. I have started to analyze images and am documenting the signal areas for the images in the ATTR vs Control samples in:

+ **Isaiah Jimenez -> Hiroki ATTR & Control Images** . By finding the differences in the signal area (percent of image with Amyloids present) in the sample of the patients with ATTR and the sample of the patients without it, we can find the percentage values of the ATTR sample and control sample respectively to be 9.98576283% and 20.70630984% which seems to be significant. We believe the antibody in this stain was binding randomly so we don't believe we can find any significant results so we discarded this.



This week I completed all of the following certifications to be able to access patient data and go into the wet lab (except for EHS-PROG-2700):

All student interns must complete:

EHS 4200: General Safety, Injury Prevention (IIPP) and Emergency Preparedness

PRIV-20: HIPAA/Protecting Patient Privacy 2021

Other required online courses might include; verify with supervisor if required:

EHS-PROG-1600 Bloodborne Pathogens

If the student is working with human blood or [other potentially infectious material](#) (OPIM), they must complete the University's online Blood borne Pathogen (BBP) training course.

EHS-PROG-2700 DOT: Shipping Dangerous Biological Goods or Dry Ice

EHS-1500 Biosafety

EHS-1900 Chemical Safety for Laboratories (required of all interns working in chemistry labs)

EHS-2200 Compressed Gas Safety

EHS-5275 Working Safely near Radioactive Materials

Here is the final data that was collected from the image analysis.

Amyloidosis Samples

Sample	Signal Area	Total Area	Signal Area Percent	Notes
ATTR01-1 (Skip)	-	-	-	50 nanometers scale for all, 2146x2146, 4605316 Total Pixels in each image. This is only the signal area for the red channel (amyloids) in the overall image. I utilized the make binary function in ImageJ and then calculated the signal of white compared to black, which would be considered background.
ATTR01-2	460797	4605316	10.00576291	46026635.29
ATTR01-3	403278	4605316	8.756793236	
ATTR01-4	1165559	4605316	25.30899074	
ATTR01x10 -1	778670	4605316	16.90806885	
ATTR08-1	237725	4605316	5.161969342	
ATTR08-2	86485	4605316	1.877938452	
ATTR08-3	196955	4605316	4.276688071	
ATTR08-4	516274	4605316	11.21039251	
ATTR08x10 -1	133580	4605316	2.900561004	
ATTR09-1	374226	4605316	8.125957046	

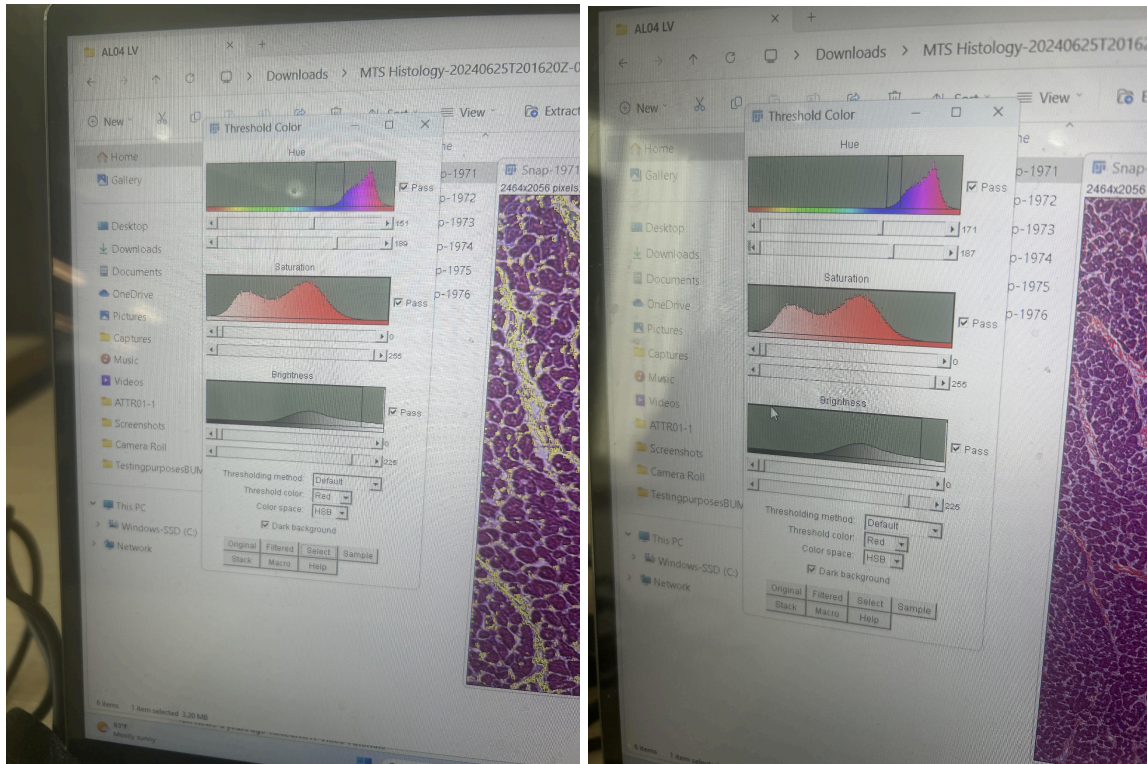
ATTR09-2	268799	4605316	5.836711314	
ATTR09-3	513614	4605316	11.15263317	
ATTR09-4	536839	4605316	11.65694167	
ATTR09x10-1	1117808	4605316	24.27212378	
ATTR09x10-2	107530	4605316	2.334910351	
		Average :	9.98576283	

Control Samples

Sample	Signal Area	Total Area	Signal Area Percent	Notes
HC04-1	25977	4605316	0.5640655278	50 nanometers scale for all, 2146x2146, 4605316 Total Pixels in each image
HC04-2	1396973	4605316	30.3339228	
HC04-3	1496636	4605316	32.49800882	
HC04x10-1	1794603	4605316	38.96807515	
HC07-3	53766	4605316	1.16747689	
		Average:	20.70630984	

Week 2: 06/24/2024

This week I continued to analyze images with ImageJ. I analyzed all the available images in the MTS Histology folder which contained samples with amyloid light chain (AL), amyloid transthyretin (ATTR) and healthy controls (HC). After importing all the images into ImageJ, I use Image -> Color Threshold and set the right threshold values for calculating just the blue signal area, which would be the area of the fibril and then the whole area by calculating blue and red signals, which would be the area of the heart tissue and the fibrils.



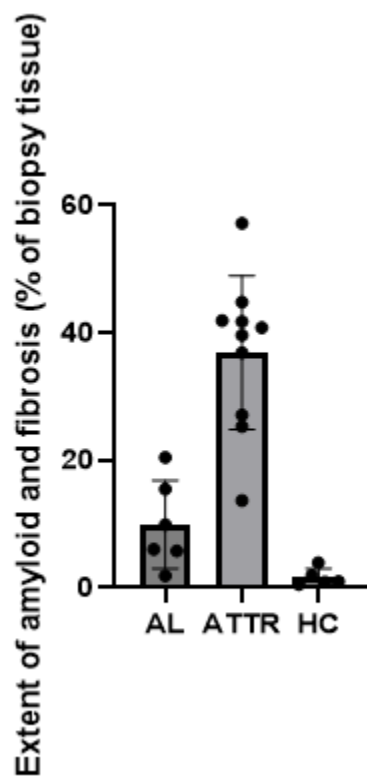
The first image above are the color threshold values for extracting the blue while the second image is the values for extracting the blue and red.

✚ MTS Histology

Sample	Signal Area (Blue)	Total Area (Blue + Red)	Signal Area Percent
AL04-1971	261176	4334450	6.025585714
AL04-1972	254036	4404182	5.768063173
AL04-1973	868088	4253260	20.40994437
AL04-1974	551763	3568068	15.46391493
AL04-1975	439215	4437475	9.897858579
AL04-1976	87734	4699031	1.867065784
ATTR01-1675	1688578	4048108	41.71277051
ATTR01-1676	1720677	4219560	40.77858829

ATTR01-1677	1797103	4012780	44.78448856
ATTR01-1678	938214	3709095	25.2949574
ATTR01-1679	579063	4234487	13.67492686
HC04-1719	24556	4552275	0.5394225964
HC04-1720	46274	4679481	0.9888703469
HC04-1721	41335	4648255	0.8892584421
HC04-1722	151179	3873417	3.902987982
HC04-1723	87062	4411430	1.97355506
SATTR01-1724	1162713	2939656	39.55268916
SATTR01-1725	1314188	3137099	41.89182426
SATTR01-1726	1150763	4252801	27.05894304
SATTR01-1728	2326070	4070663	57.14228861
SATTR03-1729	1531282	4158563	36.82238312

Here is the data that was collected.



Differences appear to be statistically significant in the difference between AL, ATTR and HC patients ($p < .001$).

I completed **TTR-KO Mouse Genotyping**. The genotyping protocol has been designed to identify the unknown genotype in the mouse strain B6;129S6.

Working Solutions

50mM NaOH Solution - This solution is made by adding 250 μ L of 10M NaOH to 50 mL of distilled water (ddH₂O).

1X TAE Buffer - This is prepared by diluting a 50X TAE Stock solution at a 1:50 ratio.

Procedure

1. DNA Digestion

Step 1: Add 300 μ L of the 50mM NaOH solution to your sample.

Step 2: Heat the mixture at 95°C for 30 minutes. This step helps break down the DNA for further analysis.

Step 3: Add 50 μ L of 1M Tris-buffer at pH 8. This neutralizes the solution.

Step 4: Vortex the PCR tubes to mix the contents thoroughly.

2. PCR Amplification

PCR (Polymerase Chain Reaction) is a technique used to amplify DNA, making millions of copies of specific DNA sequence.

Step 1: Create Adjusted Master Mix

10 μ L of 2X Green Dream Taq Mix (contains Taq polymerase, dNTPs, and buffer).

0.5 μ L of 10 μ M oIMR8334 (Forward primer).

0.5 μ L of 10 μ M oIMR8335 (Primer 1).

0.5 μ L of 10 μ M oIMR8336 (Primer 2).

5.5 μ L of ddH₂O (distilled water).

Multiply these volumes by (n + 3), where n is the number of DNA samples you have.

Step 2: Add 17 μ L of the Master Mix to each PCR tube.

Step 3: Add 3 μ L of your DNA sample to each PCR tube for a total volume of 20 μ L.

Step 4: Vortex the tubes, centrifuge them briefly to collect the contents at the bottom, and place them in the thermocycler (a machine that amplifies DNA by cycling through different temperatures).

Thermocycler Program:

95°C for 5 minutes

94°C for 30 seconds

60°C for 30 seconds

72°C for 60 seconds

72°C for 5 minutes

The steps from 94°C to 72°C are repeated 33 times to amplify the DNA.

We completed this gel electrophoresis and got samples for the DNA to do the gel electrophoresis to make sure that the right DNA is present within the sample.

I have been continuing to:

- Taking images with the microscope to find areas that potentially have amyloids and analyzing the signal area to see how much amyloids or fibrils are present within the sample.
- Did a PCR yesterday to amplify the mutant ATTR mouse DNA.
- Labeling test tube.
- Shadowing Isaiah and Nixuan

Potential School report ideas:

- If a patient has a high amount of fibrils (high risk of amyloid light chain or transthyretin amyloidosis)
- Amyloid deposition on histology
- Is mitochondrial function correlated to amyloids- histological differences cardiomyocytes vs controls
- Are there validated ways of validating mitochondrial function normal vs abnormal - scaling system
- Automation or being able to do it faster for the classifier

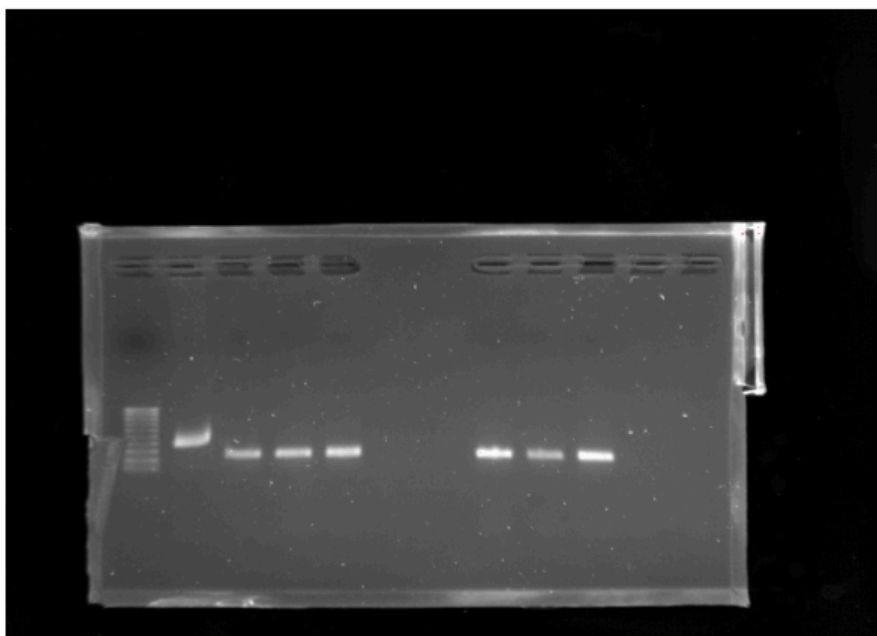
In the next coming months virtually, I could :

- a. try to create a classifier to be used with Qupath for differentiating between ATTR and healthy heart tissue
- b. Continue to calculate signal area of microscopic heart tissue images
- c. Continue working on BUMP research paper and potential literature review

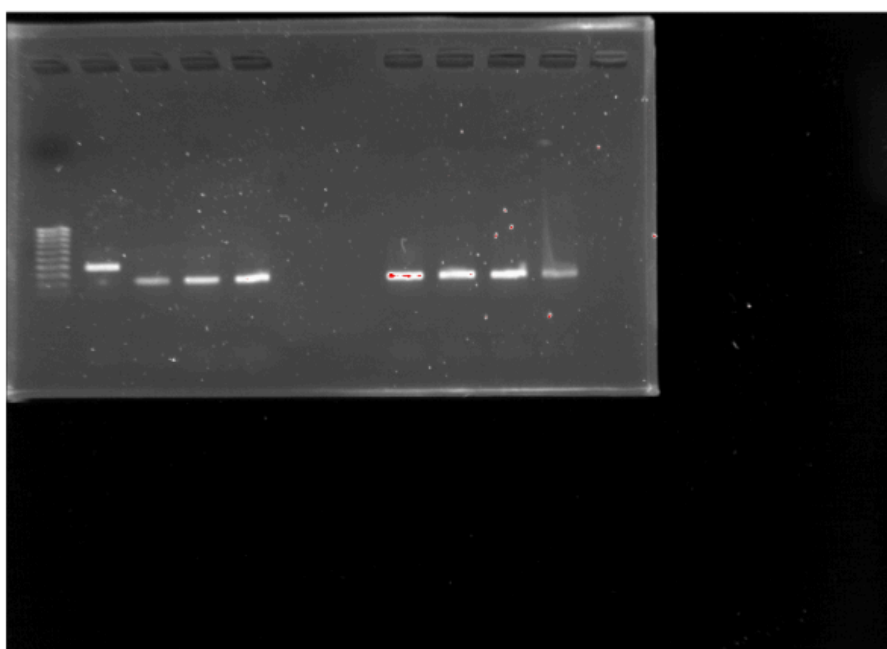
Week 3: 07/01/2024

This week I will redo the gel electrophoresis with the extra samples that we had. This would allow us to hopefully see DNA bands and be able to make a solid conclusion as to whether the target proteins are present. We completed the full gel electrophoresis procedure along with Mr. Jimenez and were able to get a successful image.

Aarush_MouseGenotype_7-1-24(2)



Aarush_MouseGenotype_7-1-24



Location: C:/Users/CVI user/Desktop/Isaiah_____

6/28/24 Gel Electrophoresis

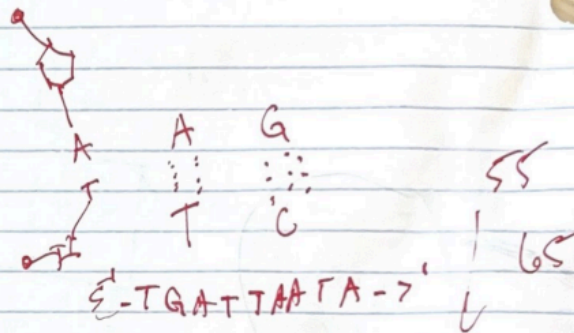
6/27/24 PCR

Top	WT F	60N L	57N R	59N N	57N L	58N L	56N L	
Bottom (orange)	WT F	60N R	58N R	57N N	58N RR	58N LR	56N R	58N N
mastermix	X18							

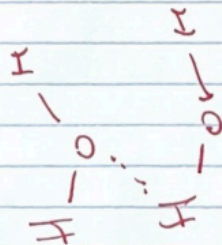
180 ML
9
9
9
9

50 ML
2.5
2.5
2.5
27.5

ACT A G
T C



1) 3'-ACTAATTA-5'



2) 3'-GCCGGC-5'
5'-CGACCGTATA-3'

3) 3'-CGGG-



Mr. Jimenez Lab Presentation:  6-mon Mouse Congo Red Histology.pptx

My lab presentation for the week:  LabMeeting7-3-2024.pptx

Dr. Alexander Lab Meeting topics of discussion:

1. Putting hold on BUMP project due to other teammate not showing interest in research paper - rather get it into a conference publication. Any good conferences?
2. New experiment and research paper can discern amyloid (ATTR & AL) and healthy control (HC) from samples' signal area in imageJ. Would it be fine to get it as a preprint when it is finished since the data was collected in the lab or are you planning on using that data for your own research paper?
3. My school is doing an internship check and I gave the necessary details to Mr. Kitakata because he filled out the form for communicating with my teacher
4. Letter of recommendation with Mr. Kitakata and Isaiah help

Think about clinical implication

Central lines?

Subcutaneous setting

Monitoring and delivery

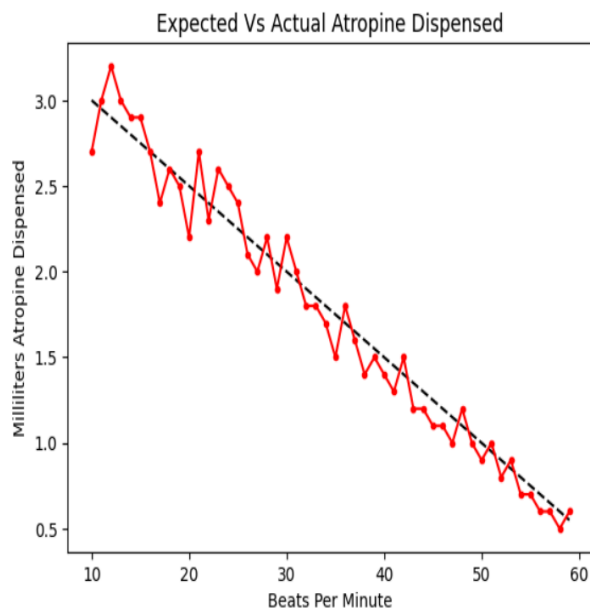
Leverage current wearables – apple watch?

Week 4: 07/08/2024

I am working on MTS analysis for male and female heart tissue samples. I have decided to start working alongside my partner for a research paper by converting the BUMP poster into a full research paper with Ethan Gracias.

Here is the results that I completed in the research paper:

“To evaluate the performance of BUMP, we measured the accuracy of the Atropine dosage, which in this scenario was substituted for water, at heart rates ranging from 10 beats per minute to 59 beats per minute. The data collected has included the beats per minute recorded by a potentiometer rather than the actual beats per minute sensed by the BPM sensor. This is due to the Arduino PulseSense Heart Rate Sensor’s inability to accurately read the heart rate of the user, which will be improved in future prototypes. Additionally, the use of a heart rate sensor for testing would require the human subject to have a BPM below 59 BPM which would constitute a medical emergency for the patient, thus we utilized a potentiometer to manually set the heart rate to be tested. Using the equation $f(x) = .05x + 3.5$ with $f(x)$ being the amount of atropine to be dispensed in milliliters and x being the heart rate, we can take the readings.”



Here is the original poster:

[BUMP: A Novel Atropine Pump for patients with Crashing Bradycardia via Continuous B...](#)

Full research paper (in-progress):

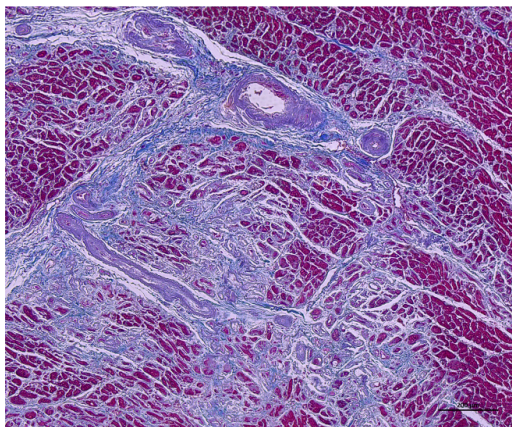
[BUMP: A Novel Atropine Pump for patients with Crashing Bradycardia via Continuous B...](#)

I have also begun work on the MTS analysis for mice and am following a streamlined process using ImageJ with the same color settings to yield results that can be quantified.

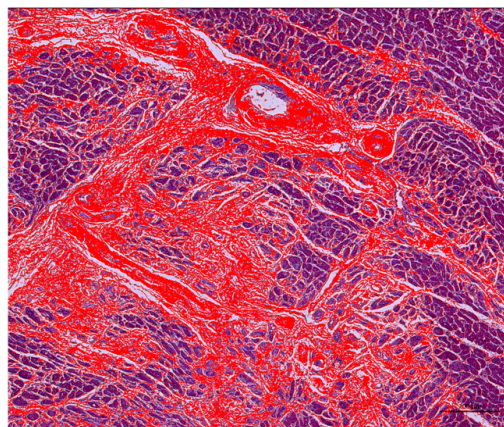
Here is the process I used to quantify the fibrosis in each image I was given:

Step #1-2

Post-imaging quantification of fibrosis



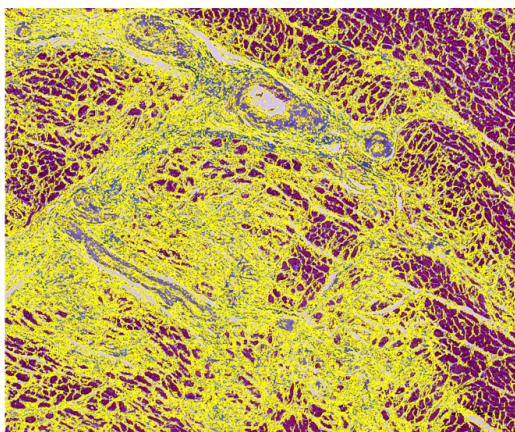
Amyloid Patient (AL)
Left Ventricle



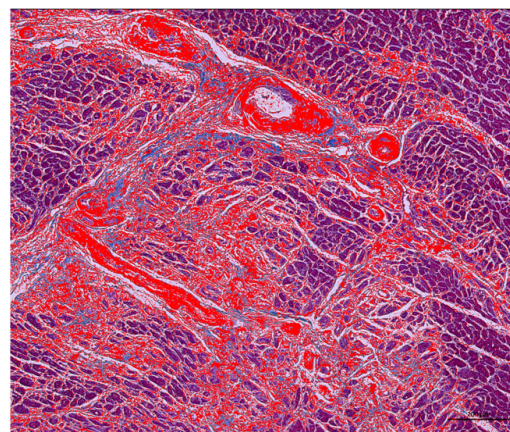
Threshold Color Hue: 171-187
Full Saturation
Brightness: 0-225

Step #3-4

Post-imaging quantification of fibrosis



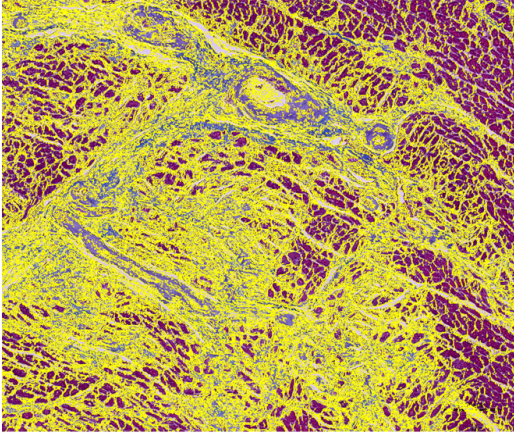
Select & measure pixel area



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

Step #5

Post-imaging quantification of fibrosis



Select & measure pixel area
Blue (1,333,787) + Red = 1,954,097 pixels

- $\frac{\text{Blue Channel Area}}{(\text{Blue} + \text{Red Channel Area})} * 100 = \% \text{ Fibrosis}$
- $\frac{1,333,787}{1,954,097} * 100 = 68.3$
- Repeated for:
 1. AL04
 2. ATTR01
 3. HC04

Week 5: 07/15/2024

I am continuing to work on MTS analysis for male and female heart tissue samples. My school requires that I complete a research paper relevant to the topic of internship which is due on the first day of school. So I have chosen my topic for the research paper.

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

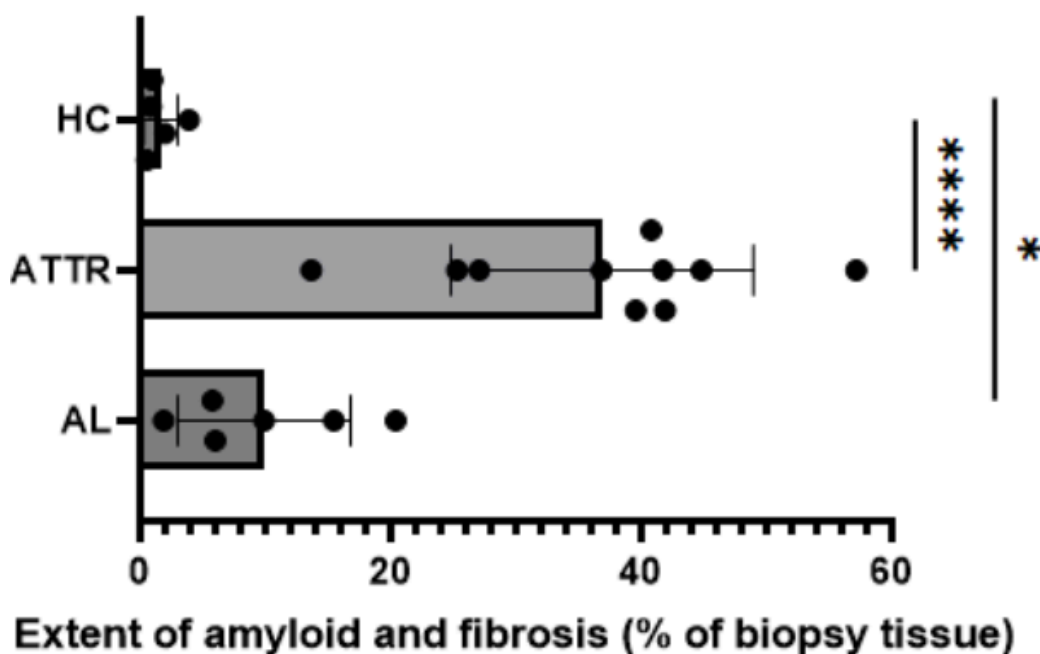
For this experiment, I will be using data that was previously collected from the human data of ATTR, AL and Healthy Control.

✚ MTS Histology

Looking at the data, there is a significant difference between Amyloid light chain, Amyloid transthyretin and healthy control with respect to the signal area percent.

Sample	Signal Area Percent Average
Amyloid Light Chain	9.9%
Amyloid Transthyretin	36.8%
Healthy Control	1.6%

Doing the analysis and flipping the previous graph we see that the differences in signal area percent is statistically significant.



The 1 asterisks indicates that Healthy Control vs Amyloid Light Chain $p < .05$

The 4 asterisks indicate Healthy Control vs Amyloid Transthyretin $p < .0001$. Since these results are statistically significant, Dr. Alexander has advised me to continue this imaging with the sample mice and then if the results are statistically significant there, the data can potentially make its way into a current research paper.

Week 6: 07/22/2024

This week I finished the entirety of the female mice MTS analysis. The data is seen below:

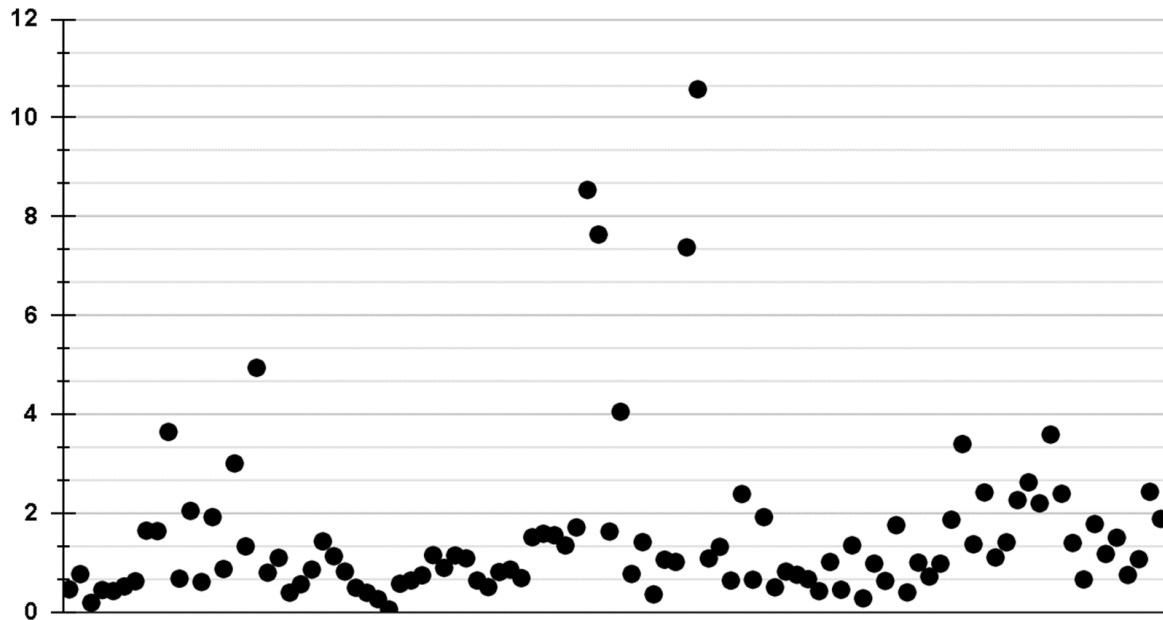
MTS Mice Aarush

Sample	Signal Area (Blue)	Total Area (Blue + Red)	Signal Area Percent
F-015D-L-880	13663	2971482	0.4598042324
F-015D-L-881	38783	5051924	0.7676877166
F-015D-L-882	7621	4051780	0.1880901727
F-015D-L-883	22550	5055672	0.4460336826
F-015D-L-884	17222	4043861	0.425880118
F-015D-L-885	26219	5056926	0.5184770353
F-015D-L-886	21766	3495304	0.6227212283
F-015D-L-887	83289	5055578	1.647467411
F-015D-L-888	80198	4893705	1.638799233
F-015D-L-889	184196	5052952	3.64531466
F-015D-R-890	31577	4664818	0.6769181563
F-015D-R-891	103543	5056322	2.047792842
F-015D-R-892	27442	4504867	0.6091633782
F-015D-R-893	97030	5048938	1.921790285
F-015D-R-896	29416	3376790	0.8711231673
F-015D-R-897	151876	5050080	3.007397903
F-015D-R-898	50676	3811948	1.329399037
F-015D-R-899	249748	5052832	4.942733105
F-015D-R-901	38858	4869389	0.7980056635
F-015D-R-902	55393	5046333	1.097688163
F-024D-L-837	14240	3622131	0.3931387352
F-024D-L-838	28430	5050865	0.562873884
F-024D-L-839	43321	5038207	0.8598495457
F-024D-L-840	72238	5048594	1.430853818
F-024D-L-841	41287	3654571	1.129735884
F-024D-L-842	31886	3888741	0.8199568961
F-024D-L-843	19380	3936970	0.4922567355
F-024D-L-845	15783	4039445	0.3907219927
F-024D-L-846	11433	4358036	0.2623429453
F-024D-L-847	2463	4644219	0.05303367477
F-024D-R-848	20205	3519746	0.5740471045
F-024D-R-849	23899	3741075	0.6388270751
F-024D-R-850	25099	3375065	0.7436597517
F-024D-R-852	39499	3428877	1.151951499

F-024D-R-853	32163	3594716	0.8947299314
F-024D-R-854	40094	3499542	1.145692779
F-024D-R-855	39857	3665532	1.087345575
F-024D-R-856	26681	4175959	0.6389191082
F-024D-R-857	21370	4222290	0.5061234543
L_Female-090D-1-10x	30330	3744690	0.8099468848
L_Female-090D-1-20x	34803	4048413	0.8596701967
L_Female-090D-2-10x	22819	3319535	0.6874155567
L_Female-090D-2-20x	57731	3812215	1.514368943
L_Female-090D-3-10x	62139	3919721	1.585291402
L_Female-090D-3-20x	67621	4348979	1.554870695
L_Female-090D-4-10x	48271	3579371	1.3485889
L_Female-090D-4-20x	59763	3489342	1.712729793
L_Female-090D-5-10x	178993	2095394	8.542212109
L_Female-090D-5-20x	209376	2740675	7.639577841
R_Female-090D-6-10x	67118	4118252	1.629769135
R_Female-090D-6-20x	171341	4227983	4.052547042
R_Female-090D-7-10x	33323	4317589	0.7717964818
R_Female-090D-7-20x	61082	4314666	1.415683161
R_Female-090D-8-10x	14757	4121964	0.3580089491
R_Female-090D-8-20xa	43694	4121964	1.060028666
R_Female-090D-8-20xb	40818	4023846	1.014402639
R_Female-090D-9-10x	159735	2164462	7.379893941
R_Female-090D-9-20x	240385	2272460	10.57818399
R_Female-090D-10-10x	40863	3764818	1.085391113
R_Female-090D-10-20x	50950	3860420	1.319804581
F-284D-L-860	19839	3110808	0.6377442774
F-284D-L-861	120607	5048786	2.388831691
F-284D-L-862	23310	3559665	0.6548369018
F-284D-L-863	97022	5049831	1.921292019
F-284D-L-864	17868	3570175	0.5004796684
F-284D-L-865	41390	5052797	0.8191502647
F-284D-L-866	27235	3607133	0.7550317662
F-284D-L-867	33735	5059874	0.6667162068
F-284D-L-868	14508	3419304	0.4242968745
F-284D-L-869	51315	5060773	1.013975533

F-284D-R-870	13699	3029934	0.4521220594
F-284D-R-871	68229	5051971	1.350542194
F-284D-R-872	9648	3468797	0.2781367719
F-284D-R-873	49544	5048378	0.981384516
F-284D-R-874	23779	3773738	0.6301179361
F-284D-R-875	88952	5058882	1.758333165
F-284D-R-876	14759	3705124	0.3983402445
F-284D-R-877	50642	5057370	1.001350504
F-284D-R-878	26485	3682209	0.7192693299
F-284D-R-879	49602	5061902	0.9799083428
L_Female-603D-1-10x	67206	3596108	1.868853772
L_Female-603D-1-20x	123207	3624166	3.399595935
L_Female-603D-2-10x	49466	3605636	1.371907758
L_Female-603D-2-20x	88794	3667990	2.420780864
L_Female-603D-3-10x	43928	3974529	1.105237879
L_Female-603D-3-20x	55822	3953311	1.412031586
L_Female-603D-4-10x	84157	3714405	2.265692621
L_Female-603D-4-20x	103233	3933019	2.624777556
L_Female-603D-5-10x	84651	3846675	2.200627815
L_Female-603D-5-20x	147310	4101547	3.591571668
R_Female-603D-6-10x	87328	3646402	2.394908735
R_Female-603D-6-20x	50679	3624284	1.398317571
R_Female-603D-7-10x	24408	3697161	0.6601822317
R_Female-603D-7-20x	68177	3827816	1.781093971
R_Female-603D-8-10x	41795	3553828	1.176055791
R_Female-603D-8-20x	52316	3476009	1.505059394
R_Female-603D-9-10x	30586	4072155	0.7511010755
R_Female-603D-9-20x	44863	4191272	1.070391041
R_Female-603D-10-10x	94970	3901459	2.434217558
R_Female-603D-10-20x	81476	4320284	1.885894538

Signal Area Percent Among Female Mice



The average signal area percent among these samples is 1.54% and despite not having the key to which samples are ATTR vs Healthy control, it is likely that all these samples are Healthy control due to the low signal area percent.

Lab Meeting Topics of discussion:

1. Mr. Kitakata meeting with teacher - Journal is shared with you in google drive
2. Currently working on Same image analysis with MTS Mice
3. I know we briefly talked about this earlier but now that I have all 300 images of mice is it okay to start making the classifier?

Week 7: 07/29/2024

This week I finished the entirety of the 300 mice MTS analysis. The male data that I finished this week is seen below:

L_Male-123D-1-10x	31940	4124720	0.7743555926
L_Male-123D-1-20x	24461	4230172	0.5782507189
L_Male-123D-2-10x	13945	4405103	0.3165646751
L_Male-123D-2-20x	9444	4500585	0.2098393875
L_Male-123D-3-10x	15457	4186570	0.3692043845
L_Male-123D-3-20x	39545	4141788	0.9547808821
L_Male-123D-4-10x	17429	4176095	0.4173516168
L_Male-123D-4-20x	36665	4285345	0.85559039
L_Male-123D-5-10x	36950	3975080	0.9295410407
L_Male-123D-5-20x	64600	3658277	1.765858627
R_Male-123D-6-10x	19353	4128854	0.4687257045
R_Male-123D-6-20x	27047	4208707	0.6426439284
R_Male-123D-7-10x	100419	4260794	2.356814246
R_Male-123D-7-20x	22634	4558712	0.4964998886
R_Male-123D-8-10x	11984	4428837	0.2705902249
R_Male-123D-8-20x	10038	4500457	0.2230440153
R_Male-123D-9-10x	63260	3655334	1.730621607
R_Male-123D-9-20x	55097	3346343	1.646483938
R_Male-123D-10-10x	40413	4161688	0.9710723149
R_Male-123D-10-20x	66092	3982218	1.659678099
L_Male-268D-1-10x	31538	2585209	1.219940051
L_Male-268D-1-20x	52056	4024206	1.293571949
L_Male-268D-2-10x	29288	4098178	0.7146590509
L_Male-268D-2-20x	48694	4282022	1.137173046
L_Male-268D-3-10x	327891	2245017	14.60527916
L_Male-268D-3-20xa	418133	2903927	14.39888124
L_Male-268D-3-20xb	220082	3895888	5.649084368
L_Male-268D-4-10x	20113	3808385	0.5281241261
L_Male-268D-4-20x	55125	4185888	1.316924868
L_Male-268D-5-10x	32670	3840440	0.8506837758
L_Male-268D-5-20x	41245	4119875	1.001122607
R_Male-268D-6-10x	16809	4067795	0.4132214136
R_Male-268D-6-20x	14748	4354912	0.3386520784
R_Male-268D-7-10x	21475	3856372	0.5568705509

R_Male-268D-7-20x	19569	4152228	0.4712891489
R_Male-268D-8-10x	45510	3845241	1.183540902
R_Male-268D-8-20x	31978	3967802	0.805937393
R_Male-268D-9-10x	21623	4150063	0.521028235
R_Male-268D-9-20x	30463	4399784	0.6923748984
R_Male-268D-10-10x	16640	3989002	0.4171469455
R_Male-268D-10-20x	13515	4470602	0.3023082797
L_Male-372D-1-10x	35901	3712147	0.9671222611
L_Male-372D-1-20x	22155	3725380	0.5947044328
L_Male-372D-2-10x	30923	4272407	0.7237840402
L_Male-372D-2-20x	18590	4160579	0.4468128114
L_Male-372D-3-10x	24477	4054920	0.6036370631
L_Male-372D-3-20x	12477	4124314	0.3025230378
L_Male-372D-4-10x	8905	4411157	0.2018744742
L_Male-372D-4-20x	40231	3982451	1.010207031
L_Male-372D-5-10x	63888	3901691	1.637443867
L_Male-372D-5-20x	57617	3973988	1.449853397
R_Male-372D-6-10x	32150	4130000	0.7784503632
R_Male-372D-6-20x	53142	3987157	1.332829382
R_Male-372D-7-10x	13951	4315167	0.3233015084
R_Male-372D-7-20x	46827	4069877	1.150575312
R_Male-372D-8-10x	19406	4118419	0.4712002348
R_Male-372D-8-20x	50294	4039090	1.245181464
R_Male-372D-9-10x	17350	4170858	0.4159815558
R_Male-372D-9-20x	16252	4351456	0.3734841855
R_Male-372D-10-10x	18572	4160143	0.4464269618
R_Male-372D-10-20x	29401	4218290	0.6969885902
L_Male-558D-1-10x	77525	3835162	2.021426996
L_Male-558D-1-20x	56109	4189595	1.339246395
L_Male-558D-2-10x	19894	5065984	0.3926976477
L_Male-558D-2-20x	21048	4051648	0.5194923152
L_Male-558D-3-10x	28989	3943081	0.7351865204
L_Male-558D-3-20x	34965	4089773	0.8549374256
L_Male-558D-4-10x	16627	4066734	0.4088538862
L_Male-558D-4-20x	40149	3897685	1.030072979
L_Male-558D-5-10x	13663	4223509	0.3234987779
L_Male-558D-5-20x	12673	4260222	0.2974727608
R_Male-558D-6-10x	20896	3869304	0.5400454449

R_Male-558D-6-20x	25967	3856449	0.6733396448
R_Male-558D-7-10x	24313	3870201	0.6282102661
R_Male-558D-7-20x	42293	4038263	1.047306726
R_Male-558D-8-10x	24462	4254325	0.5749913324
R_Male-558D-8-20x	32178	4299102	0.7484818923
R_Male-558D-9-10x	77247	3382947	2.283423299
R_Male-558D-9-20x	107412	3687593	2.912794335
R_Male-558D-10-10x	30002	4031307	0.7442251359
R_Male-558D-10-20x	51554	4246569	1.214015362
L_Male-588D-1-10x	12213	4333468	0.2818297031
L_Male-588D-1-20x	16997	4333209	0.3922497161
L_Male-588D-2-10x	20473	3813541	0.5368501348
L_Male-588D-2-20x	26967	4057206	0.664669233
L_Male-588D-3-10x	11745	3962386	0.2964123132
L_Male-588D-3-20x	33776	4082856	0.8272640524
L_Male-588D-4-10x	49685	3931131	1.263885635
L_Male-588D-4-20x	64254	4169044	1.541216643
L_Male-588D-5-10x	22518	3755984	0.5995233207
L_Male-588D-5-20x	79454	3800424	2.090661463
R_Male-588D-6-10x	44580	4304458	1.035670461
R_Male-588D-6-20x	24487	4344117	0.5636818714
R_Male-588D-7-10x	43737	4175199	1.047542884
R_Male-588D-7-20x	37781	4262399	0.886378774
R_Male-588D-8-10x	24028	3945318	0.6090256856
R_Male-588D-8-20x	23230	4211219	0.5516217513
R_Male-588D-9-10x	36564	3851147	0.9494314291
R_Male-588D-9-20x	70692	3585288	1.971724447
R_Male-588D-10-10x	61380	3850489	1.594083245
R_Male-588D-10-20x	19570	4124453	0.4744871623
L_Male-607D-1-10x	29742	3888851	0.7648017371
L_Male-607D-1-20x	59460	3692864	1.610132407
L_Male-607D-2-10x	42681	3858671	1.106106222
L_Male-607D-2-20x	50396	3854971	1.307299069
L_Male-607D-3-10x	12496	4148006	0.3012531804
L_Male-607D-3-20x	6656	4286158	0.1552905889
L_Male-607D-4-10x	17844	4190422	0.425828234
L_Male-607D-4-20x	33572	3979277	0.8436708477
L_Male-607D-5-10x	14586	4101851	0.3555955592

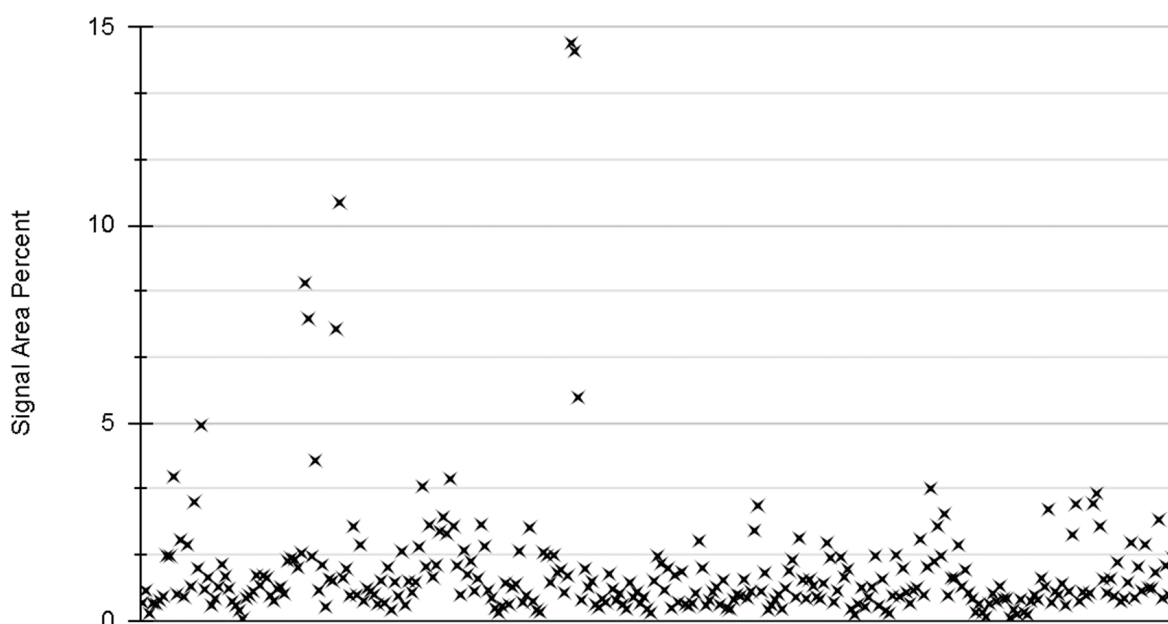
L_Male-607D-5-20x	23541	3946974	0.5964315954
R_Male-607D-6-10x	34043	3977194	0.8559552287
R_Male-607D-6-20x	63548	3867418	1.643163475
R_Male-607D-7-10x	15838	4134514	0.3830679978
R_Male-607D-7-20x	41311	3905947	1.057643639
R_Male-607D-8-10x	11528	4410453	0.2613790466
R_Male-607D-8-20x	8578	4527441	0.1894668534
R_Male-607D-9-10x	28000	4412071	0.6346226069
R_Male-607D-9-20x	68215	4097718	1.664707039
R_Male-607D-10-10x	27418	4439594	0.6175789948
R_Male-607D-10-20x	55466	4175108	1.32849258
L_Male-780D-1-10x	29245	4056673	0.7209109534
L_Male-780D-1-20x	19679	4432596	0.4439610558
L_Male-780D-2-10x	33212	4290032	0.7741667195
L_Male-780D-2-20x	33715	4024610	0.837720922
L_Male-780D-3-10x	84166	4085168	2.060282466
L_Male-780D-3-20x	26833	4082200	0.6573171329
L_Male-780D-4-10x	50633	3716298	1.362458016
L_Male-780D-4-20x	125917	3759255	3.349520051
L_Male-780D-5-10x	51584	3439817	1.49961466
L_Male-780D-5-20x	82763	3453322	2.396619834
R_Male-780D-6-10x	64626	3933393	1.643008975
R_Male-780D-6-20x	107299	3968158	2.704000194
R_Male-780D-7-10x	25069	3905339	0.6419161051
R_Male-780D-7-20x	44420	4085866	1.087162428
R_Male-780D-8-10x	39719	3663214	1.084266439
R_Male-780D-8-20x	69109	3604691	1.917196231
R_Male-780D-9-10x	33844	3901069	0.8675570722
R_Male-780D-9-20x	52781	4098275	1.287883317
R_Male-780D-10-10x	27846	3930017	0.7085465534
R_Male-780D-10-20x	22754	4086849	0.5567614561
L_Male-789D-1-10x	9482	4363419	0.2173066579
L_Male-789D-1-20x	18160	4231987	0.4291128494
L_Male-789D-2-10x	9325	4466455	0.2087785503
L_Male-789D-2-20x	4648	4611626	0.1007887457
L_Male-789D-3-10x	18502	4273172	0.4329804651
L_Male-789D-3-20x	28513	4119287	0.6921828948
L_Male-789D-4-10x	20437	4128398	0.4950346357

L_Male-789D-4-20x	34614	3945827	0.8772305527
L_Male-789D-5-10x	24093	4261701	0.5653376433
L_Male-789D-5-20x	24231	4278956	0.5662829905
R_Male-789D-6-10x	3442	4393252	0.07834742919
R_Male-789D-6-20x	12716	4484786	0.2835363828
R_Male-789D-7-10x	7311	4410913	0.1657479982
R_Male-789D-7-20x	22953	4216531	0.5443574351
R_Male-789D-8-10x	9547	4380138	0.2179611693
R_Male-789D-8-20x	7181	4543388	0.1580538576
R_Male-789D-9-10x	22052	4426914	0.4981348181
R_Male-789D-9-20x	27780	4247050	0.6541010819
R_Male-789D-10-10x	22815	4262235	0.5352825454
R_Male-789D-10-20x	44451	4118000	1.079431763
L_Male-796D-1-10x	33815	3913072	0.8641548124
L_Male-796D-1-20x	110733	3929166	2.818231655
L_Male-796D-2-10x	20573	4456817	0.4616074656
L_Male-796D-2-20x	32655	4424692	0.738017471
L_Male-796D-3-10x	27407	4153676	0.6598251765
L_Male-796D-3-20x	39201	4163196	0.9416083221
L_Male-796D-4-10x	17500	4447373	0.3934907191
L_Male-796D-4-20x	33303	4477318	0.743815829
L_Male-796D-5-10x	85696	3932940	2.178929757
L_Male-796D-5-20x	124932	4231884	2.952160314
R_Male-796D-6-10x	21335	4287167	0.4976479806
R_Male-796D-6-20x	31691	4350257	0.7284856964
R_Male-796D-7-10x	27154	4255729	0.6380575455
R_Male-796D-7-20x	31077	4434955	0.7007286432
R_Male-796D-8-10x	117753	3972731	2.964031544
R_Male-796D-8-20x	140061	4346765	3.222189375
R_Male-796D-9-10x	103176	4313916	2.391701646
R_Male-796D-9-20x	45192	4311412	1.048194884
R_Male-796D-10-10x	30408	4321526	0.7036403345
R_Male-796D-10-20x	45941	4314030	1.064920735
L_Male-881D-1-10x	26171	4203733	0.6225657053
L_Male-881D-1-20x	61197	4134261	1.480240362
L_Male-881D-2-10x	19993	4100765	0.4875431779
L_Male-881D-2-20x	28838	5065984	0.5692477513
L_Male-881D-3-10x	37472	3845427	0.9744561527

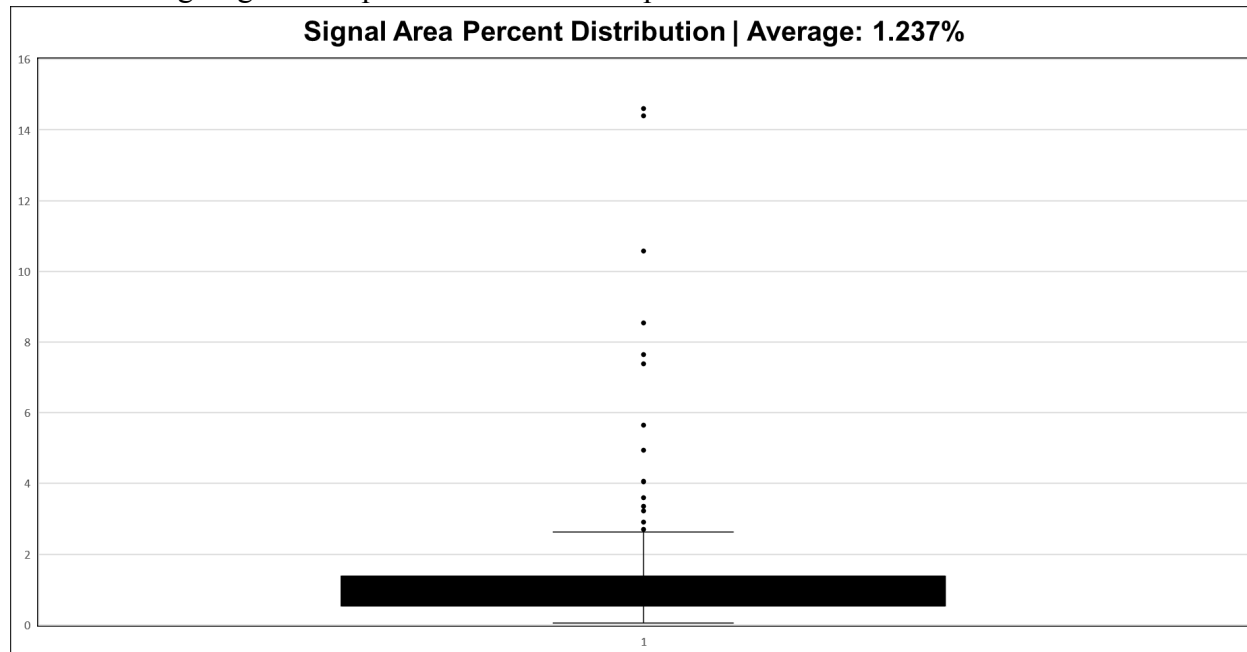
L_Male-881D-3-20x	72925	3689289	1.976668133
L_Male-881D-4-10x	24686	4204938	0.5870716762
L_Male-881D-4-20x	56887	4163239	1.366412065
L_Male-881D-5-10x	29286	3844780	0.7617080821
L_Male-881D-5-20x	71792	3721446	1.929142597
R_Male-881D-6-10x	31736	3809374	0.8331027618
R_Male-881D-6-20x	31930	3883394	0.8222189147
R_Male-881D-7-10x	45912	3745510	1.225787676
R_Male-881D-7-20x	95626	3739515	2.557176532
R_Male-881D-8-10x	23613	4112051	0.5742389868
R_Male-881D-8-20x	55164	3955776	1.394517789
R_Male-881D-9-10x	27401	4227104	0.648221572
R_Male-881D-9-20x	65622	4139431	1.585290345
R_Male-881D-10-10x	18514	4154508	0.4456364027
R_Male-881D-10-20x	27007	4192651	0.6441509203

Here is a full representation of all the 300 samples in a scatterplot with each X representing one sample's signal area.

Signal Area Percent Among All Samples



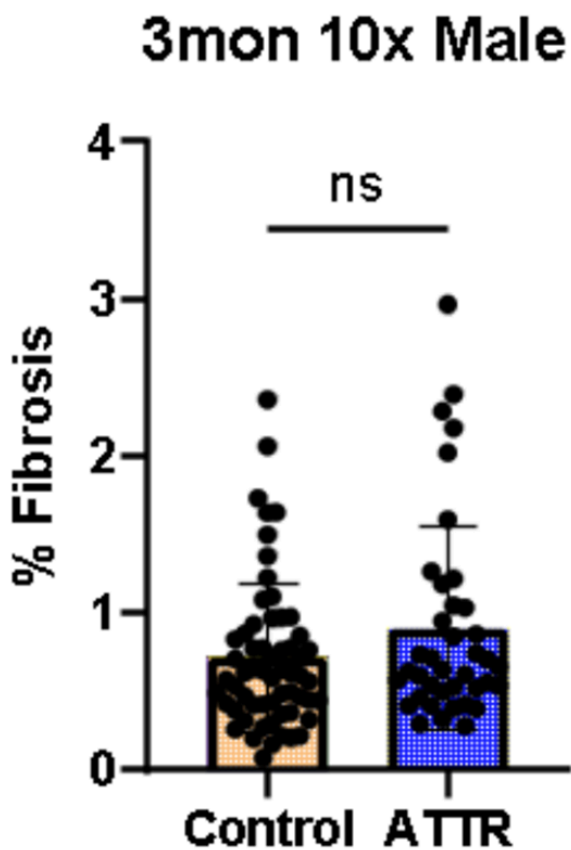
We can also visualize the distribution of the signal area percent throughout the samples to see that the average signal area percent across all samples was 1.237%



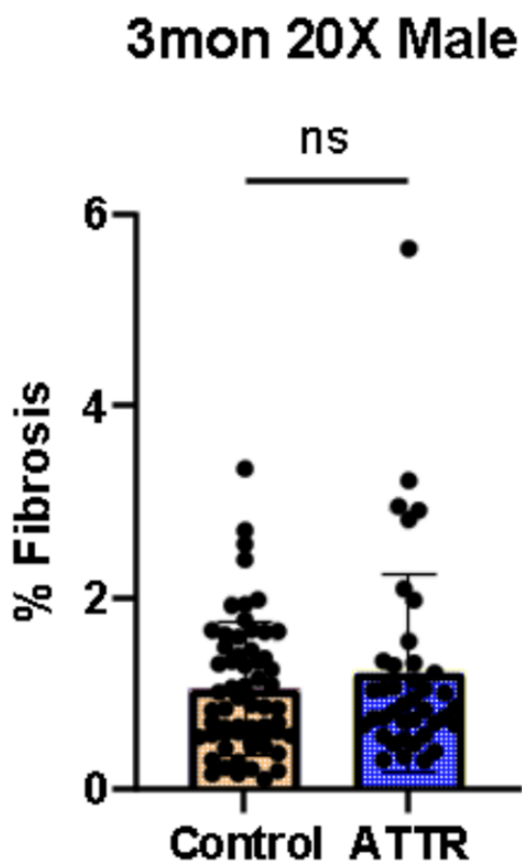
Week 8: 08/05/2024

Now that I've completed all the data analysis with all of the samples, I can now create visual representations of the data.

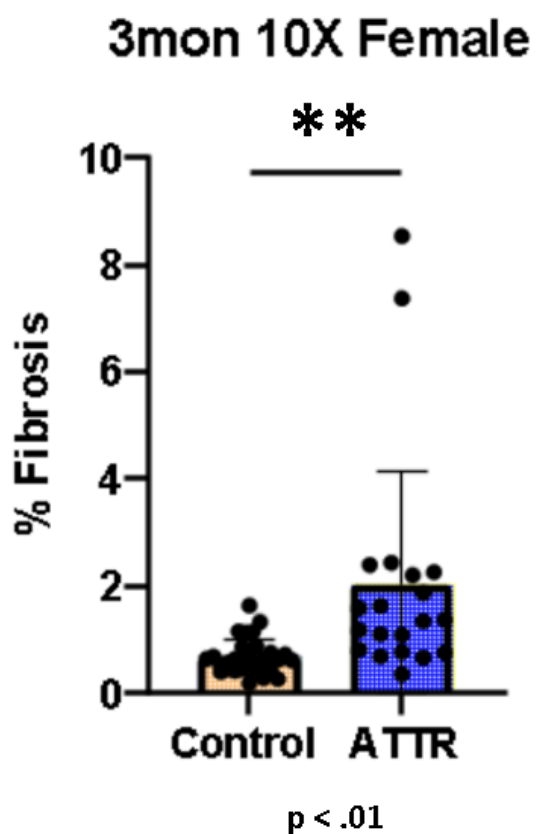
Here is the data analysis comparing male vs control amyloid transthyretin mice.



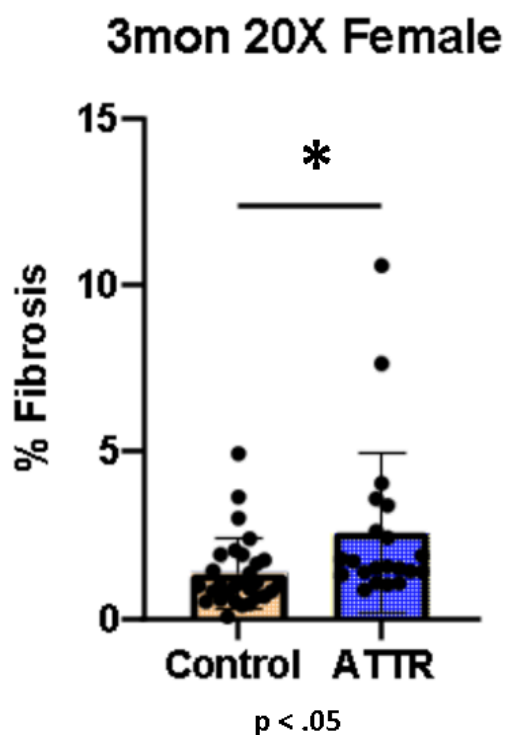
Parameter	Value
Test	Unpaired t test
P value	0.1046
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.638, df=97
Mean of column A	0.7136
Mean of column B	0.8981
Difference between means (B - A) ± SEM	0.1844 ± 0.1126
95% confidence interval	-0.03903 to 0.4079
R squared (eta squared)	0.02692
F test to compare variances	
F, DFn, Dfd	1.917, 38, 59
P value (F test)	0.0241
P value summary (F test)	*
Significantly different (P < 0.05)?	Yes
Sample size, column A	60
Sample size, column B	39



Parameter	Value
Test	Unpaired t test
P value	0.3438
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.9512, df=98
Mean of column A	1.05
Mean of column B	1.214
Difference between means (B - A) ± SEM	0.1645 ± 0.1730
95% confidence interval	-0.1787 to 0.5078
R squared (eta squared)	0.009148
F test to compare variances	
F, DFn, Dfd	2.246, 39, 59
P value (F test)	0.0049
P value summary (F test)	**
Significantly different (P < 0.05)?	Yes
Sample size, column A	60
Sample size, column B	40

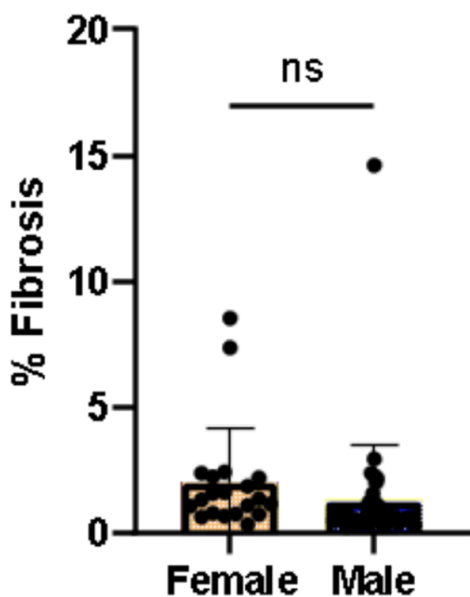


Parameter	Value
Test	Unpaired t test
P value	0.0014
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.398, df=48
Mean of column A	0.6821
Mean of column B	2.021
Difference between means (B - A) ± SEM	1.339 ± 0.3942
95% confidence interval	0.5468 to 2.132
R squared (eta squared)	0.1939
F test to compare variances	
F, DF _n , DF _d	41.97, 19, 29
P value (F test)	<0.0001
P value summary (F test)	****
Significantly different (P < 0.05)?	Yes
Sample size, column A	30
Sample size, column B	20



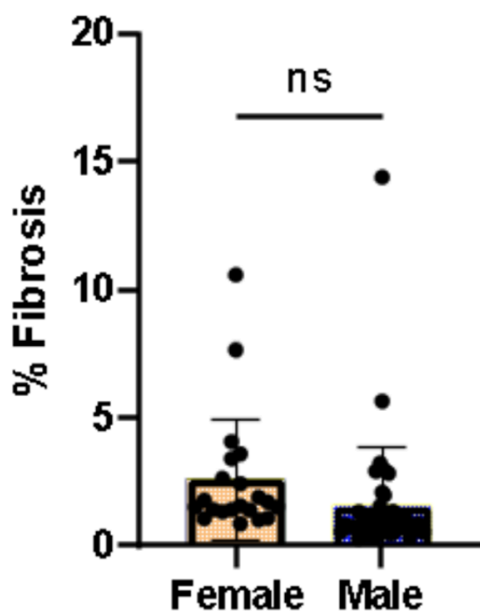
Parameter	Value
Test	Unpaired t test
P value	0.0196
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.416, df=48
Mean of column A	1.355
Mean of column B	2.562
Difference between means (B - A) ± SEM	1.207 ± 0.4996
95% confidence interval	0.2024 to 2.212
R squared (eta squared)	0.1084
F test to compare variances	
F, DFn, Dfd	5.072, 20, 28
P value (F test)	0.0001
P value summary (F test)	***
Significantly different (P < 0.05)?	Yes
Sample size, column A	29
Sample size, column B	21

10X Male vs. Female ATTR



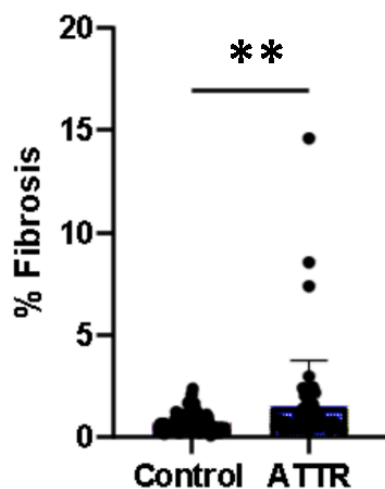
Parameter	Value
Test	Unpaired t test
P value	0.2041
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.284, df=58
Mean of column A (Female)	2.021
Mean of column B (Male)	1.241
Difference between means (B - A) ± SEM	-0.7806 ± 0.6077
95% confidence interval	-1.997 to 0.4359
R squared (eta squared)	0.02766
F test to compare variances	
F, DFn, Dfd	1.124, 39, 19
P value (F test)	0.8062
P value summary (F test)	ns
Significantly different (P < 0.05)?	No
Sample size, column A (Female)	20
Sample size, column B (Male)	40

20X Male vs. Female ATTR



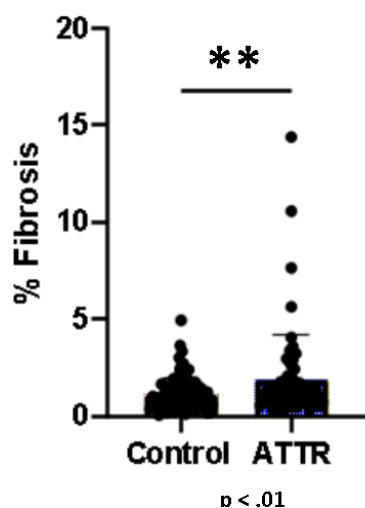
Parameter	Value
Test	Unpaired t test
P value	0.106
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.641, df=60
Mean of column A (Female)	2.562
Mean of column B (Male)	1.536
Difference between means (B - A) ± SEM	-1.027 ± 0.6255
95% confidence interval	-2.278 to 0.2245
R squared (eta squared)	0.04297
F test to compare variances	
F, DFn, Dfd	1.081, 20, 40
P value (F test)	0.808
P value summary (F test)	ns
Significantly different (P < 0.05)?	No
Sample size, column A (Female)	21
Sample size, column B (Male)	41

Combined 10X ATTR vs. Control



Parameter	Value
Test	Unpaired t test
P value	0.106
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.641, df=60
Mean of column A (Female)	2.562
Mean of column B (Male)	1.536
Difference between means (B - A) ± SEM	-1.027 ± 0.6255
95% confidence interval	-2.278 to 0.2245
R squared (eta squared)	0.04297
F test to compare variances	
F, DFn, Dfd	1.081, 20, 40
P value (F test)	0.808
P value summary (F test)	ns
Significantly different (P < 0.05)?	No
Sample size, column A (Female)	21
Sample size, column B (Male)	41

Combined 20X ATTR vs. Control



Parameter	Value
Test	Unpaired t test
P value	0.0077
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.701, df=149
Mean of column A (Control)	1.149
Mean of column B (ATTR)	1.884
Difference between means (B - A) ± SEM	0.7342 ± 0.2718
95% confidence interval	0.1970 to 1.271
R squared (eta squared)	0.04667
F test to compare variances	
F, DFn, Dfd	7.962, 61, 88
P value (F test)	<0.0001
P value summary (F test)	****
Significantly different (P < 0.05)?	Yes
Sample size, column A (Control)	89
Sample size, column B (ATTR)	62

Using this data we can conclude that:

Mice with Amyloid Transthyretin have a greater signal area of fibrosis based on our MTS analysis with female attr mice depicting a greater signal area compared to female control mice.

Week 9: 08/12/2024

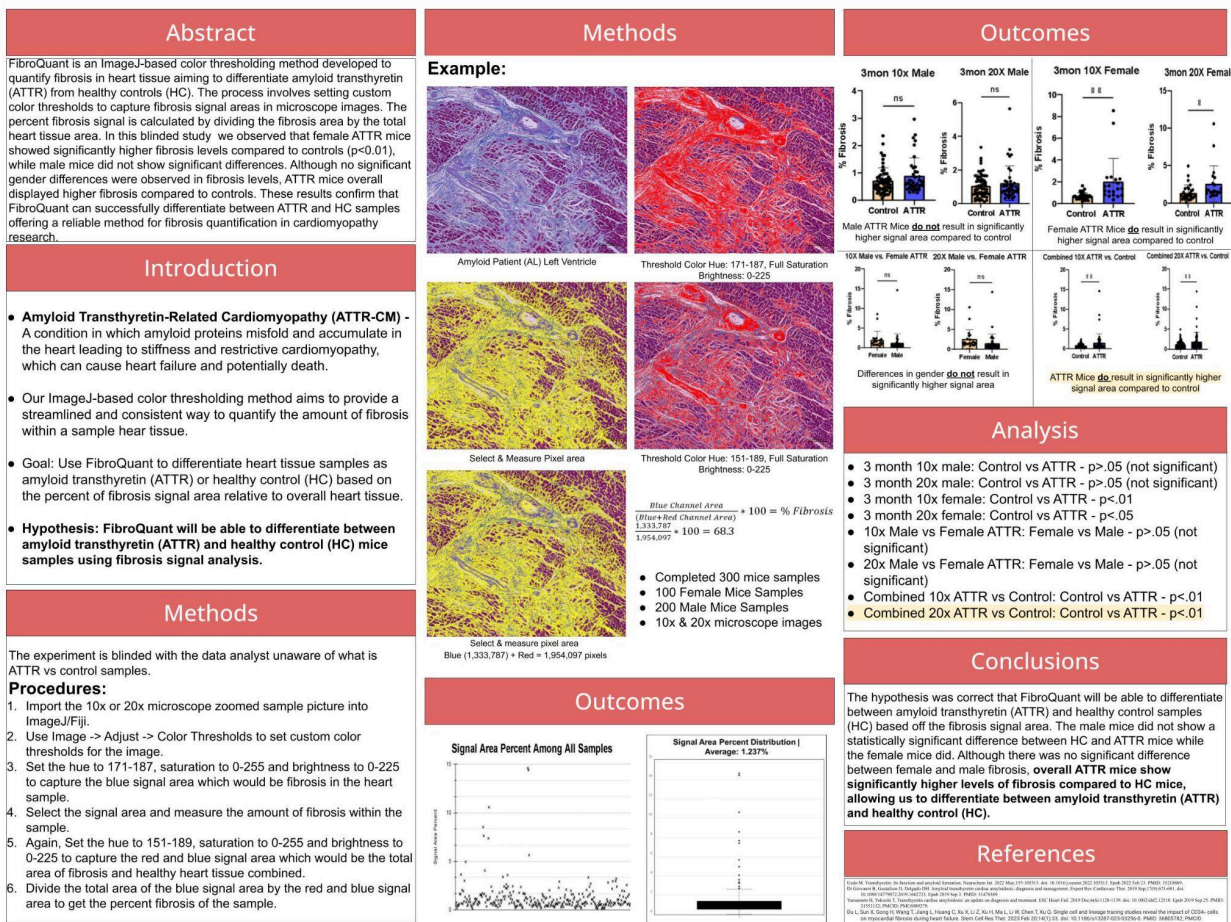
This is my final week of the internship. I'm grateful for the opportunity given by Dr. Alexander to learn and conduct research in his lab.

Here is the final poster that I've finished for this week.

FibroQuant: ImageJ-Based Color Thresholding to Differentiate Amyloid Transthyretin fro...

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

Aarush Kandukoori | Kevin Alexander, Hiroki Kitakata | Stanford Alexander Lab



Synopsis

- Developed FibroQuant: ImageJ-Based Color Thresholding to Differentiate Amyloid Transthyretin from Healthy Controls via Fibrosis Signal Analysis and found a statistically significant difference ($p < .01$) in fibrosis of 300 control and ATTR mice samples, validating the method's accuracy.
- Completed TTR-KO Mouse Genotyping and successfully saw bands for downstream experiments



