Module 2:

Team Members:

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Project Title:

Idiopathic Pulmonary Fibrosis: Computational Assessment of Fibrotic Lesions in a Bleomycin-Induced Mouse Lung Model

Project Goal:

This project seeks to to develop an image analysis pipeline that will predict the extent of fibrosis in the lung at different biopsy depths from the top of the lung. Through this analysis, the project will answer the following questions:

1. How does the extent of fibrosis vary throughout the depth of a fibrotic lung?
2. Using interpolation, can you predict the amount of fibrosis at a specific depth into the lung?

Disease Background:

- Prevalence & incidence (in the U.S.)
    - Prevalance: ~250,000 people in the United States currently living with Idiopathic Pulmonary Fibrosis (IPF) (varies by source, but tends to be in the all around the same range).
        - Source: https://www.pulmonaryfibrosis.org/understanding-pff/about-pulmonary-fibrosis/what-is-pulmonary-fibrosis
    - Incidence: ~50,000 new cases every year within the United States.
        - Source: https://www.pulmonaryfibrosis.org/understanding-pff/about-pulmonary-fibrosis/what-is-pulmonary-fibrosis
- Risk factors (genetic, lifestyle)
    - Genetic
        - Many familial studies have identified rare and

common genetic variants associated with both familial interstitial pneumonia (FIP). These variants include the single nucleotide polymorphism (SNP) in the MUC5B gene as well as genes related to innate immune function (TOLLIP, TLR3, IL1RN, IL8, TGFB1), and epithelial barrier function (DSP, DPP9). Additional gene ontologies represented in those identified variants include telomere maintenance (TERT, TERC, OBFC1, TINF2, DKC1, RTEL1, PARN), surfactant production (SFTPC, SFTPA2, ABCA3), and cell cycle regulation (KIF15, MAD1L1, CDKN1A).

- MUC5B Promoter Variant
  - The rs35705950 variant in the promoter region of the mucin 5B (MUC5B) gene was first identified in a 2011 genome-wide linkage study and is associated with an approximately 7-fold increased risk of developing IPF.
  - Known to be the most significant genetic risk factor in developing IPF
  - The minor, disease-associated T allele at rs35705950 is a gain-of-function variant has been suggested to drive differential methylation of and transcription factor binding to the MUC5B leading to increased MUC5B expression.
  - In a study done:
    - MUC5B

expression was uniformly increased in lungs of IPF patients compared to controls, regardless of whether the MUC5B SNP was present
        - Increased numbers of MUC5B expressing cells have been detected in the distal airways of IPF patients
    - Source: https://pmc.ncbi.nlm.nih.gov/articles/PMC7801923/ and https://publications.ersnet.org/content/erj/45/6/1717
- Lifestyle
    - Age
        - The risk of developing IPF increases as you get older. IPF is diagnosed most often in people who are in their 60s or 70s.
    - Lifestyle Habits
        - Smoking is a common risk factor among people who have IPF.
            - A small 2017 study found that current smokers developed IPF at a younger age than both nonsmokers and former smokers.

        - People who have a history of smoking can also develop COPD, which is a group of progressive lung diseases that can be associated with pulmonary fibrosis.
    - Environment
        - Studies have identified a significantly increased risk of IPF with exposure to inorganic and animal dust, and chemical fumes; includes:
            - wood dust and use of wood fires, metal dusts, such as brass, lead, and steel, stone dust and stone polishes, smoke, vegetable dust, livestock dust, asbestos, bird droppings, pesticides, mold, and soil dust
    - Gender
        - Male gender is a common risk factor for IPF (The prevalence of interstitial lung disease is 20% higher in men than in women)
            - In a study of 920 patients with IPF, 62% were men
    - Source: https://www.healthline.com/health/managing-idiopathic-pulmonary-fibrosis/lifestyle-risk-factors#The-takeaway and https://pro.boehringer-ingelheim.com/us/insights-in-ild/diseases/risk-factors-ipf
- Symptoms
    - Common symptoms
        - Shortness of Breath: A symptom that gets worse over time. At first, you may be short of breath only during exercise. Over time, you may feel breathless even at rest.
        - A dry cough that gets worse. You may have

repeated bouts of coughing that you cannot control.
        - Aching muscles and joints; Clubbing , which is a widening and rounding of the tips of the fingers or toes; Extreme tiredness; Gradual, unintended weight loss; Generally feeling unwell; Rapid, shallow breathing
    - Secondary Symptoms
        - The strain IPF can put on your lungs and heart increases the risk of secondary conditions such as:
            - Chest infections and airway infections, for example bronchitis, pneumonia and influenza (flu)
            - Pulmonary hypertension
            - Right sided heart failure
    - Source: https://www.nhlbi.nih.gov/health/idiopathic-pulmonary-fibrosis/symptoms
- Standard of care treatment(s)
    - Past treatment
        - Until 2014:
            - The standard practice for treating IPF focused primarily on immunosuppressant therapy using a combination of prednisone, azathioprine, and N-acetylcysteine. However, after the release of the results from the Evaluating the Effectiveness of Prednisone, Azathioprine and N-acetylcysteine in Patients with IPF (PANTHER-IPF) TRIAL in 2012, it became apparent that, compared with placebo, this form of treatment actually increased the likelihood of hospitalization, treatment-related severe adverse events, and death in IPF patients.
    - Current Treatment
        - Antifibrotic therapies
            - Pirfenidone and nintedanib

        - Pirfenidone is a modified pyridine small molecule with antifibrotic, anti-inflammatory and antioxidant properties. It decreases the production of collagen, slows the fibrotic process by suppressing the cytokine TGF-β and lowers the rate of decline in FVC
        - Nintedanib is an intracellular tyrosine kinase inhibitor that binds to adenosine triphosphate binding sites, thus suppressing the signaling pathways linked to vascular endothelial growth factor receptor, fibroblast growth factor receptor 1–3, and platelet-derived growth factor receptor α and β. These effects on receptor tyrosine kinases lead to decreased fibroblast activity
        - The two drugs are able to slow deterioration in FVC and provide the patient with greater comfort for a noticeably longer time.
    - Lung Transplantation
        - Lung transplantation may be a viable treatment option for IPF. A key advantage compared with other modes of treatment is that it is the only method to improve both symptoms and survival time.

        - Potential Future Treatment
            - Stem cells
                - A nonpharmacological treatment being explored for used in IPF is based on the finding that mesenchymal stem cells (MSC), multipotent, undifferentiated cells, can regulate fibrotic processes and exert control over lung injury and repair.
        - Source: https://pmc.ncbi.nlm.nih.gov/articles/PMC9060042/
- Biological mechanisms (anatomy, organ physiology, cell & molecular physiology)
    - Anatomy
        - In IPF, the normal lung structure composed of alveoli, surrounded by delicate interstitial tissue and capillaries, becomes progressively distorted. The lower lobes and subpleural regions are most affected, where alveolar walls thicken, airspaces collapse, and fibrotic scars form. Honeycomb cysts, visible on imaging and histology, represent clusters of destroyed alveoli replaced by fibrotic tissue. This remodeling reduces lung elasticity and surface area for gas exchange, giving the lung a stiff, shrunken appearance and making breathing increasingly labored.
        - Source: https://www.physio-pedia.com/Lung_Anatomy and https://www.ncbi.nlm.nih.gov/books/NBK448162/
    - Organ Physiology
        - At the organ level, IPF severely disrupts pulmonary mechanics and gas exchange. The progressive fibrosis decreases lung compliance, meaning greater effort is required for each breath. The thickened alveolar-capillary barrier impairs oxygen diffusion, leading to hypoxemia and shortness of breath during exertion. Over time, chronic hypoxia and increased pulmonary vascular resistance can cause pulmonary hypertension and right ventricular strain. The

restrictive physiology, characterized by reduced total lung capacity and vital capacity, is a big feature that doctors observe in pulmonary function tests.
        - Source: https://pmc.ncbi.nlm.nih.gov/articles/PMC9489199/
    - Cell and Molecular Physiology
        - Cellular dysfunction in IPF centers on the abnormal activation of alveolar epithelial cells and fibroblasts. Repeated microinjury to type I alveolar cells triggers aberrant repair signaling, including epithelial–mesenchymal transition (EMT). Type II alveolar cells proliferate excessively but fail to properly regenerate the epithelium. Fibroblasts and myofibroblasts accumulate and deposit excessive extracellular matrix (ECM) proteins such as collagen and fibronectin, forming fibrotic foci. This loss of epithelial integrity and uncontrolled fibroblast activity creates a self-perpetuating cycle of scarring and stiffness=progressively worse IPF.
        - On the molecular scale, IPF involves dysregulation of several key signaling pathways. Transforming growth factor-β (TGF-β) is the principal profibrotic mediator, stimulating fibroblast differentiation and collagen synthesis. Other pathways, including platelet-derived growth factor (PDGF), Wnt/β-catenin, and connective tissue growth factor (CTGF), contribute to fibroblast proliferation and resistance to apoptosis. Oxidative stress, telomere shortening, and mitochondrial dysfunction in epithelial cells exacerbate tissue injury. The imbalance between matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) further promotes ECM accumulation, driving irreversible fibrosis.
        - Source: https://pmc.ncbi.nlm.nih.gov/articles/PMC9952569/

Data-Set:

Filenames and Depths Data Set:

- How it was collected/methodology:
    - Mice were injected with Bleomycin which is an antibody that has been isolated from a fungus.
        - It is typically used in chemotherapy to treat cancer, however, it also causes lung fibrosis.
            - Researchers are able to use this to their advantage to study the effects of lug fibrosis in mice.
        - Three weeks after injection, the mice were humanely killed and their lungs were harvested. The lungs were then mounted in a gel and thinly sliced along the transverse plane.
            - Next, they were placed on a microscope slide and immunostain was added. Immunostain binds to the protein of interest and illuminates the effected areas in white. However, it also highlights blood vessels but these can be identified because they typically appear in circles.
        - Images of numerous lungs, each at different depths, were captured.
            - Using these we are able to analyze the data and find a correlation between depth in the lung and degree of lung fibrosis.
- Overall Content:
    - The filenames and depths data set lists all the filenames for the 78 black and white images collected at different depths into a fibrotic mouse lung. Each file name has its corresponding image number within it, and provides the depth from lung surface (in micrometers).
        - white = fibrotic lesion; black = healthy lung
- Pathological/Physiological Processes:
    - The data set reflects both physiological and pathological

processes such as pulmonary fibrosis, extracellular matrix (ECM) remodeling, fibroblast activation, collagen deposition, and the loss of normal alveolar structure that alter tissue stiffness and impair lung function.
        - Source: https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2023.1205924/full (for physiological effects of pulmonary fibrosis)
- Units:
    - Depth: in micrometers
- Bias
    - One potential bias of the data set is that the selected image regions were not uniformly distributed across the lung, meaning areas with visibly higher or lower fibrosis may have been unintentionally overrepresented. This could introduce sampling bias and could skew the results toward showing a stronger or weaker fibrosis-depth relationship than what actually exists.
- Assumptions and Limitations:
    - It's assumed that all the depths recorded in the data-set is true and that all procedures in collecting the data were performed accurately and honestly.
    - One limitation is that the data set included only a limited number of image slices taken at discrete depths, which may not fully capture the continuous variation of fibrosis throughout the entire lung. This could introduce sampling gaps rather than representing the complete depth-dependent pattern.
- Source: This data was collected from Dr. Shayne Pierce-Cottlers Lab. Her lab studies microvascular engineering; https://www.peircecottlerlab.com/

Data Analysis:

- The questions that were given to us were: (1) How does the extent of fibrosis vary throughout the depth of a fibrotic lung? (2) Using interpolation, can you predict the amount of fibrosis at a specific depth into the lung?
- To answer our second question, we conducted a linear interpolation (can also make it quadratic and cubic). To do this, we chose 6 images (out of the 78) at different depths, built a list for the images, calculated

the number of black and white pixels for each, and then interpolated a point (at an inputted depth by the operator). Using this graph, we were able to answer our first, overarching question by comparing the plot of depth of image vs percentage white pixels to the graph with the interpolated point in red.

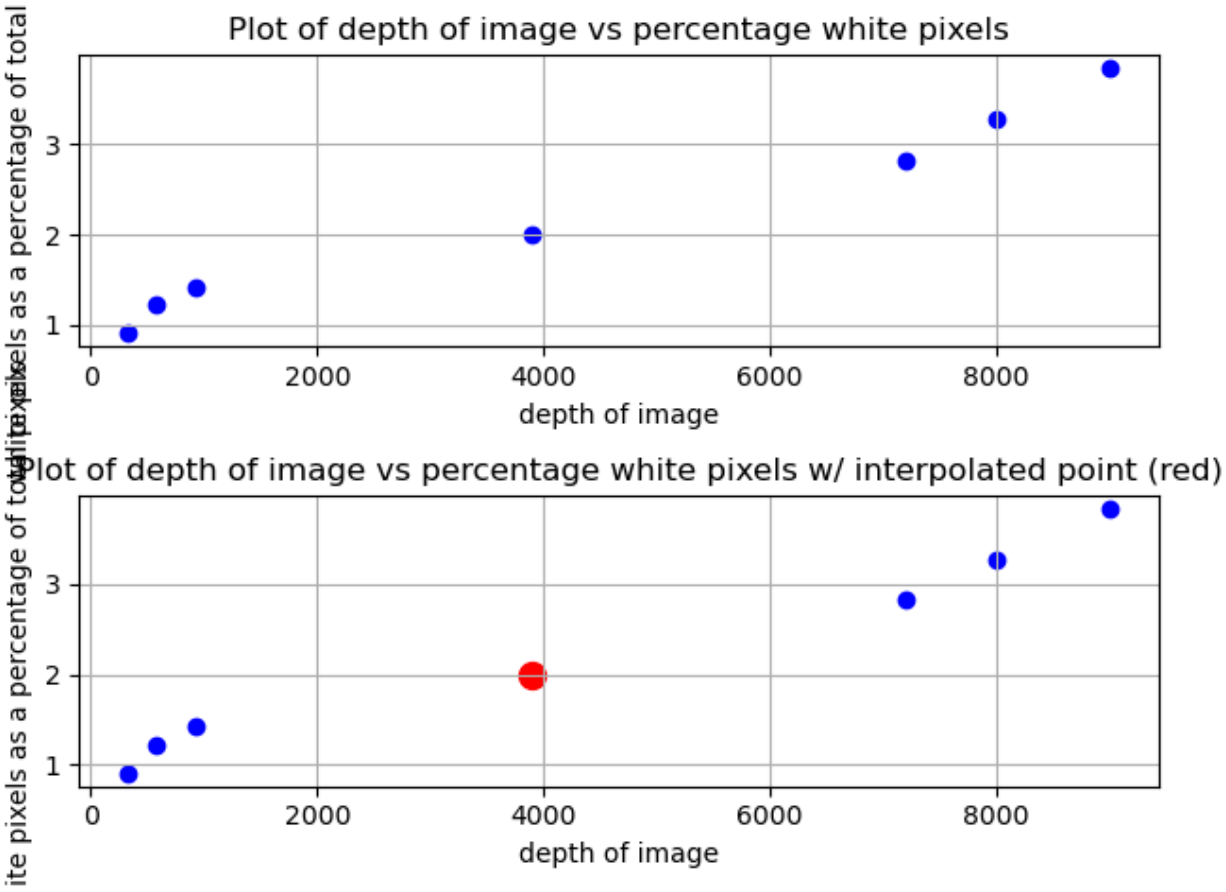
- Here's an outline of the steps we went through to develop the following code:
    - Step 1: Imported necessary libaries to handle image processing, numerical calculations, plotting, and data export
    - Step 2: Loaded image file names and depth values (in microns) of the images we wanted to analyze
    - Step 3: Read each image in grayscale and built lists for storing the image data, as well as tracking the corresponding counts of white pixels, black pixels, and their calculated percentages for later analysis
    - Step 4: Counted black and white pixels for each image chosen
    - Step 5: Calculated the percent of white pixels by computing the ratio of white pixels to total pixels and converting it to a percentage
    - Step 6: Saved results to a CSV file for record keeping and later analysis of the images we chose
    - Step 7: Perform interpolation and visualization; plotted two scatterplots to show the comparison

In [ ]:
```
'''Module 2: count black and white pixels in a .jpg and extrapolate points'''

#1: Imported necessary libaries to handle image processing, numerical calculat
from termcolor import colored
import cv2
import numpy as np
import matplotlib.pyplot as plt
from scipy.interpolate import interp1d
import pandas as pd

#2: Load the images you want to analyze

filenames = [
    r"/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule
    r"/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule
    r"/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule
    r"/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule
    r"/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule
    r"/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule
    r"/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule
```

```
]

#Enter the depth of each image (in the same order that the images are listed a
depths = [
    9000,
    570,
    7200,
    8000,
    330,
    920,
    3900
]

#3: Make the lists that will be used

images = []
white_counts = []
black_counts = []
white_percents = []

# Build the list of all the images you are analyzing
for filename in filenames:
    img = cv2.imread(filename, 0)
    images.append(img)

#4: For each image (until the end of the list of images), calculate the number
for x in range(len(filenames)):
    _, binary = cv2.threshold(images[x], 127, 255, cv2.THRESH_BINARY)

    white = np.sum(binary == 255)
    black = np.sum(binary == 0)

    white_counts.append(white)
    black_counts.append(black)

# Print the number of white and black pixels in each image.
print(colored("Counts of pixel by color in each image", "yellow"))
for x in range(len(filenames)):
    print(colored(f"White pixels in image {x}: {white_counts[x]}", "white"))
    print(colored(f"Black pixels in image {x}: {black_counts[x]}", "black"))
    print()

#5: Calculate the percentage of pixels in each image that are white and make a

for x in range(len(filenames)):
    white_percent = (100 * (white_counts[x] / (black_counts[x] + white_counts[
    white_percents.append(white_percent)

# Print the filename (on one line in red font), and below that line print the
print(colored("Percent white px:", "yellow"))
for x in range(len(filenames)):
    print(colored(f'{filenames[x]}:', "red"))
    print(f'{white_percents[x]}% White | Depth: {depths[x]} microns')
```

```
    print()

'''Write your data to a .csv file'''

#6: Create a DataFrame that includes the filenames, depths, and percentage of
df = pd.DataFrame({
    'Filenames': filenames,
    'Depths': depths,
    'White percents': white_percents
})

#Write that DataFrame to a .csv file
df.to_csv('Percent_White_Pixels.csv', index=False)

print("CSV file 'Percent_White_Pixels.csv' has been created.")

'''the .csv writing subroutine ends here'''

#7: Interpolate a point: given a depth, find the corresponding white pixel per

interpolate_depth = float(input(colored("Enter the depth at which you want to

x = depths
y = white_percents

i = interp1d(x, y, kind='linear')  # You can also use 'quadratic', 'cubic', et
interpolate_point = i(interpolate_depth)
print(colored(f'The interpolated point is at the x-coordinate {interpolate_dep

depths_i = depths[:]
depths_i.append(interpolate_depth)
white_percents_i = white_percents[:]
white_percents_i.append(interpolate_point)

# make two plots: one that doesn't contain the interpolated point, just the da
fig, axs = plt.subplots(2, 1)

axs[0].scatter(depths, white_percents, marker='o', linestyle='-', color='blue'
axs[0].set_title('Plot of depth of image vs percentage white pixels')
axs[0].set_xlabel('depth of image')
axs[0].set_ylabel('white pixels as a percentage of total pixels')
axs[0].grid(True)

axs[1].scatter(depths_i, white_percents_i, marker='o', linestyle='-', color='b
axs[1].set_title('Plot of depth of image vs percentage white pixels w/ interpo
axs[1].set_xlabel('depth of image')
axs[1].set_ylabel('white pixels as a percentage of total pixels')
axs[1].grid(True)
axs[1].scatter(depths_i[len(depths_i)-1], white_percents_i[len(white_percents_

# Adjust layout to prevent overlap
plt.tight_layout()
plt.show()
```

```
Counts of pixel by color in each image
White pixels in image 0: 161111
Black pixels in image 0: 4033193

White pixels in image 1: 51040
Black pixels in image 1: 4143264

White pixels in image 2: 118409
Black pixels in image 2: 4075895

White pixels in image 3: 137592
Black pixels in image 3: 4056712

White pixels in image 4: 38068
Black pixels in image 4: 4156236

White pixels in image 5: 59788
Black pixels in image 5: 4134516

White pixels in image 6: 83951
Black pixels in image 6: 4110353

Percent white px:
/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule/MASK_S
k658 Llobe ch010121.jpg:
3.8411855697631836% White | Depth: 9000 microns

/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule/MASK_S
k658 Llobe ch010025.jpg:
1.216888427734375% White | Depth: 570 microns

/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule/MASK_S
k658 Llobe ch010160.jpg:
2.8230905532836914% White | Depth: 7200 microns

/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule/MASK_S
k658 Slobe ch010119.jpg:
3.2804489135742188% White | Depth: 8000 microns

/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule/MASK_S
k658 Slobe ch010156.jpg:
0.9076118469238281% White | Depth: 330 microns

/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule/MASK_S
K658 Slobe ch010158.jpg:
1.4254570007324219% White | Depth: 920 microns

/Users/logan/Documents/GitHub/desktop-tutorial/New folder/FibrosisModule/MASK_S
K658 Llobe ch010065.jpg:
2.0015478134155273% White | Depth: 3900 microns

CSV file 'Percent_White_Pixels.csv' has been created.
The interpolated point is at the x-coordinate 3900.0 and y-coordinate 2.0015478
134155273.
```



Verify and validate your analysis:

Verification:

- **Ochoa et al.'s “Imaging of Murine Whole Lung Fibrosis by Large Scale 3D Microscopy”**: To verify our results that fibrosis severity increases with tissue depth and is most pronounced near the pleural surface, a paper by Ochoa et al. (2018) titled “Imaging of Murine Whole Lung Fibrosis by Large Scale 3D Microscopy” supports this observation. The study used optical clearing and multiphoton microscopy to visualize collagen organization throughout the murine lung, revealing spatial heterogeneity in collagen deposition and a clear pattern of increased fibrotic remodeling near surface regions compared to deeper parenchymal tissue. Although the paper did not directly measure how fibrosis changes from the pleural surface to the inner lung, their volumetric imaging in their study confirmed that fibrosis is not uniformly distributed and tends to be more concentrated in peripheral and subpleural areas. This is a finding that is consistent with our data showing a negative correlation between tissue depth and fibrotic

density.

- Source: https://pmc.ncbi.nlm.nih.gov/articles/PMC6441702/

Validation:

- Our data was validated by the rest of the images in the data set. To test our interpolation, we used a depth of 3900 microns. By taking image 65, which had the same depth, and adding that to our code, we were able to compare our interpolated point with the actual value of percent of white pixels at that depth. When making our graph with this new point, our interpolated point was exactly on top of the y-coordinate of the depth of 3900 microns, meaning that our prediction using the interpolated point was exactly the same as the actual percentage of white pixels. The y-coordinate of our interpolated point was 2.0015478134155273% and the y-coordinate for image 65 at a depth of 3900 microns was 2.0015478134155273%. To validate further, we tested a point at a depth of 6300 microns (image 107). The percentage of white pixels for this point was 2.389812469482422%. We then interpolated a point to the same depth and got the same percentage of white pixels at 2.389812469482422%. This shows that our interpolation was accurate and could be used to predict the percentage of white pixels for other depths.

Conclusions and Ethical Implications:

Conclusions:

- For our first question: "How does the extent of fibrosis vary throughout the depth of a fibrotic lung?"
  - Conclude that as depth increases, the extent/severity of pulmonary fibrosis increases. As distance from the pleural surface decreases, the extent of fibrosis lessens, showing a negative correlation between tissue depth and fibrotic density. This trend was demonstrated through our data, where higher depths corresponded to greater white pixel percentages, reflecting increased collagen deposition and more extensive tissue scarring near the pleural surface. Our data represents this as a depth of 330 microns, for example, has a white pixel percentage of 0.9076118469238281% while a depth of 7200 microns has a percentage of

2.8230905532836914%. This question was also further validated through interpolation, as discussed below.

- For our second question: "Using interpolation, can you predict the amount of fibrosis at a specific depth into the lung?"
    - Conclude that yes, we can predict the amount of fibrosis at a specific depth into a lung. This is shown as the interpolated point was exactly on top of the y-coordinate of the depth 3900 microns (an example depth we chose), meaning that our prediction using the interpolated point was exactly the same as the actual percentage of white pixels. The y-coordinate of our interpolated point was 2.0015478134155273% and the y-coordinate for image 65 at a depth of 3900 microns was 2.0015478134155273%, showing how accurate and reliable our interpolation method is for estimating fibrosis at specific depths. Other depths were also measured, such as 6300 microns, that further validated our point.

Ethical Implications:

- One ethical implication is the use of animal models. The study involves inducing pulmonary fibrosis in mice through Bleomycin injection, which causes inflammation and scarring of lung tissue. Although this method provides information about the progression and treatment of lung fibrosis, it can raise ethical concerns about animal welfare and the necessity of minimizing pain and distress. A second ethical implication is the handling and interpretation of this imaging data and its results. Researchers have a responsibility to ensure that data collected from animal tissues are analyzed and reported accurately, without manipulation or bias that could misrepresent the severity or distribution of fibrosis. There must be transparency in image selection, processing, and quantification to avoid overstating results. Additionally, since this research aims to model human disease, it carries an ethical responsibility to translate findings responsibly, especially to patients. Doctors should acknowledge the limitations of animal models and avoid premature generalizations about human health to prevent anxiety in patients. These implications emphasize the importance of both humane animal treatment and integrity in data analysis to uphold ethical standards within this research.

Limitations and Future Work:

Limitations:

- One limitation of our study was that we only had a set of pictures that we could choose from. Additionally, we only selected 6 images out of this which could have caused us to have very different results. In our case, our six random images were able to show a good trend, but if we had picked images that were outliers, our interpolation would have been very different. The final result was very dependent on the images that were selected. Additionally, there may have been some inaccuracy while counting the percentage of white pixels, because of the rings that appear in the images. These rings are blood vessels in the mouse of lungs that cannot be removed from the data set. This may inflate the number of white pixels and cause discrepancies in our values.

Future Work:

- In the future, we could use more images to create a graph with more data points that would make interpolating a point easier. Also, if we were able to, it would be interesting to do this with human lungs to see the difference in the depths and how it compares to the mouse depths. This may help us to draw more relevant conclusions and also see how similar mouse lungs are to humans; helping us judge the comparison between mouse lungs and human lungs. Furthermore, we could try to relate our findings to another biological factor such as RNA markers of fibrosis or Ashcroft scores. This may help us draw unique conclusions.

NOTES FROM YOUR TEAM:

Current Progress

- 10/6: Started working on the initial anaylsis of the 6 images. To start, Z created and ran a code to analyze his 6 images that he chose. His code successfully ran but noted that the outputed csv file isn't in a form of a table. While he was developing the code, Logan did the 5 background information bullet points and we shared our progress throughout the night.
- 10/7: We switched roles today and Logan analyzed his 6 images that he chose. He also ran into the same problem that the outputted csv file did

not output as a formatted table. Z reviewed the bullet points and updated himself on the background information. We reviewed our file together and made sure we had all the necessary information before we submit our first check-in.

- 10/9: After getting all the background information through our work on 10/6 and 10/7, we started interpolating our data. We used the provided code and ran a linear interpolation and we were able to output an accurate scatterplot to represent our data. We worked a little after class to finalize the code and graph necessary for the project submission.
- 10/14: We decided to split up the last few days to write the remaining sections of the project. Over break, we finalized our verification and validation section.
- 10/16: For our last day, Z decided to do limitations and future work while Logan did conclusions and ethical implications. We reviewed our final notebook and prepped it for submission.

Other Notes

- Successfully analyzed our 6 images (we both ran it separately).
    - Note that our data that was outputted was not in the form of a table, but (hopefully) in an acceptable format.
- Successfully outputted an interpolation linear graph that displayed very accurate results

QUESTIONS FOR YOUR TA:

- First Check-in Questions:
    - Is it okay that our code is not outputting a table? The output has all of the information we need and looks very similar to Dr. Peirce-Cottler's example, but we didn't know if it needed to look exactly like how hers outputted.
    - For our final submission, do we need to show linear, quadratic, and cubic results? Or do we just pick one to represent the data?
- Post Check-in Questions:
    - (No other further questions or concerns for the rest of the project)