

Module_2: Lung Fibrosis

Team Members:

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Project Title:

Predicting Lung Fibrosis at Biopsy Depths

Project Goal:

This project aims to investigate the variation in fibrosis extent throughout the depth of a fibrotic lung and use interpolation to predict the amount of fibrosis at a specific depth into the lung. The goal is to develop an image analysis pipeline that predicts the extent of fibrosis in the lung at various biopsy depths from the top of the lung to create a lung biopsy device.*

Disease Background:

- Prevalence & incidence
 - Prevalence:
 - Idiopathic Pulmonary Fibrosis (IPF) prevalence is higher in older individuals.
 - US estimates of prevalence = around 14 to 42.7 per 100,000 people.
 - Prevalence is rising globally - some regions show higher rates
 - North America (24.0-29.8 per 100,000)
 - South Korea (4.51 per 10,000 or 45.1 per 100,000).
 - Incidence:
 - US estimates of annual incidence of IPF = between 6.8 and 16.3 per 100,000 people.
 - IPF incidence is increasing globally.
- Risk factors (genetic, lifestyle)
 - Genetic:
 - Family history of pulmonary fibrosis (PF) or interstitial lung diseases (ILDs)
 - MUC5B Gene = a specific genetic variant (rs35705950) in this gene is a risk factor linked to both familial and idiopathic PF
 - Mutations in genes that maintain telomeres (e.g., TERT, TER, PARN, and RTEL1) can lead to the shortening of telomeres and increase the risk.

- Lifestyle:
 - Smoking
 - Occupational dusts (metal, wood, silica, agricultural)
 - Air pollution
 - Inhaling substances such as environmental irritants like mold spores, bacteria, or animal proteins
- Symptoms
 - Lung scarring leads to difficulty breathing and shortness of breath
 - Dry, persistent hacking/barking cough: over the counter drugs do not help
 - Fatigue
 - Chest pain
 - Thickening/rounding of the fingertips or toes or clubbing.
 - Weight loss due to difficulty breathing and decreased appetite.
 - Cyanosis (a blueish tint to the skin/lips): the lungs cannot adequately oxygenate the blood.
 - Joint pain and muscle weakness
- Standard of care treatment(s)
 - Diagnosis (a combination of these tests is used)
 - Chest X-rays can provide imagery/insight on potential scar tissue buildup
 - CT Scan- a type of scan that can create 3D structures by merging multiple image angles, useful to determine the extent of lung damage
 - Echocardiogram- uses sound waves to assess the heart, can also provide insight on the pressure of arteries around the heart and to the lung
 - Lung function tests
 - Spirometry test- breathe out into a tube, measures max air capacity of lung
 - Lung volume test- measures air capacity of lung functionally (while breathing in and out)
 - Lung diffusion test- measures oxygen/carbon dioxide exchange between lungs and blood
 - Pulse oximetry- measures oxygen saturation in blood
 - Exercise stress test- heart and lung function are measured during states of activity
 - Arterial blood gas test- a sample of blood is measured for carbon dioxide and oxygen levels
 - Tissue Biopsy- removes a lung tissue sample between the ribs in a minimally invasive surgery and is definitive, tested for IPF
 - Treatment
 - medications to slow progression
 - pirfenidone (Esbriet)

- inhibits the production of growth factors (Transforming Growth Factor-beta (TGF-beta) and Tumor Necrosis Factor-alpha (TNF-alpha)) and collagen
- nintedanib (Ofev)
 - inhibits many growth factors: latelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), and vascular endothelial growth factor receptor (VEGFR)
- Oxygen therapy to reduce shortness of breath
- Pulmonary rehabilitation program to teach you to manage your disease
- Lung Transplant for extreme cases
- Biological mechanisms (anatomy, organ physiology, cell & molecular physiology)
 - When the regulation between fibroblast proliferation and apoptosis is shifted toward less apoptosis or more fibroblast proliferation, there are more ECM producers.
 - When the balance/regulation between ECM turnover and deposition is shifted with less breakdown of ECM, there is more ECM (excess)
 - Altered mesenchymal cells and and alveolar epithelial cell injury --> accumulation of ECM and remodeling of lung architecture
 - More specifically, Altered lung mesenchymal cells and alveolar epithelial cell injury --> accumulation of ECM
 - 3 different processes
 - Cytokine specific Acute and chronic inflammation (de-emphasized)
 - TGF- β (Transforming Growth Factor Beta), IL-13, and PDGF (Platelet-Derived Growth Factor) increases fibroblast proliferation-> excess ECM
 - Macrophages and epithelial cells also release cytokines
 - Oxidative stress and signaling
 - constant epithelial injury leads to higher production of reactive oxygen species (ROS), which causes cell damage and activates TGF- β /Smad and MAPK pathways
 - oxidative stress is persisted as antioxidant defenses are impaired (leads to lower lung function)
 - coagulation proteinases and their tissue receptors
 - Protease-Activated Receptors (PARs) signal proteinases activating PAR-1 and PAR-2, linked with TGF-beta, leading to excess ECM accumulation
 - Organ physiology
 - Areas of dense fibrosis in the lungs lead to lower vessel density in these regions
 - Lung anatomy
 - 3 lobes in right (superior, middle, inferior) and 2 in left (superior and inferior)
 - alveoli is where oxygen diffuses in and carbon dioxide diffuses out

- alveolar epithelium made up of 2 pneumocytes: type 1(surface gas exchange) and type 2 (reduce surface tension and injury)
- in IPF, repeated injury to alveolar epithelium leads to bad/abnormal wound healing
- accumulation of fibroblasts and myofibroblasts--> excessive collagen and ECM and an overall thickening of alveolar walls
- Lungs stiffen and impair gas exchange, leading to the symptoms listed above.

Data-Set:

(Describe the data set(s) you will analyze. Cite the source(s) of the data. Describe how the data was collected -- What techniques were used? What units are the data measured in? Etc.)

- The dataset consists of 78 black and white images (.jpg) collected at various depths within a fibrotic mouse lung. White in the images indicates a fibrotic lesion, whereas black indicates healthy lung tissue. The images were collected from mice lungs (sliced and immunostained for desmin signal) with a Bleomycin-induced Lung Injury Model.

Data Analysis:

- We chose 6 images from the data set at varying depths (2 from lower depths, 2 from the middle range, and 2 at higher depths) to compare the extent of lung fibrosis at different tissue depths
- The code below extrapolates the depths of each 6 images, calculates the percentage (%) of white pixels (representing the extent of lung fibrosis, higher % = more fibrosis), and plots these extrapolated points in a plot of % white pixels vs. the depths for each image
- The code also then prompts the user to input a depth value, and using the input depth value using linear interpolation the point is plotted on a graph in red.
- We then used a linear regression on our data points to determine a line of best fit and calculate an R-squared value and slope to verify the relationship between lung fibrosis extent and depth of the tissue

```
In [13]: '''Module 2: count black and white pixels in a .jpg and extrapolate points'''
from termcolor import colored
import cv2
import numpy as np
import matplotlib.pyplot as plt
from scipy.interpolate import interp1d
import pandas as pd
```

```
# Load the images you want to analyze

filenames = [
    r"/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_Sk658 Llobe
    r"/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_Sk658 Llobe
    r"/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_SK658 Slobe
    r"/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_SK658 Slobe
    r"/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_SK658 Slobe
    r"/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_SK658 Slobe
    r"/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_Sk658 Llobe
]

# Enter the depth of each image (in the same order that the images are listed)

depths = [
    45,
    200,
    10000,
    8800,
    860,
    3100
]

# 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)

# For each image (until the end of the list of images), calculate the number of white and black pixels

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()

# Calculate the percentage of pixels in each image that are white and make a bar chart

white_percentages = [w / sum(white_counts) * 100 for w in white_counts]
black_percentages = [b / sum(black_counts) * 100 for b in black_counts]
```

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

# Print the filename (on one line in red font), and below that line print th

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'''

# 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'''

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

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

x = depths
y = white_percents

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

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
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='red')


```

```
axs[1].set_title('Plot of depth of image vs percentage white pixels w/ inter')
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_percent

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

Counts of pixel by color in each image

White pixels in image 0: 27561

Black pixels in image 0: 4166743

White pixels in image 1: 37799

Black pixels in image 1: 4156505

White pixels in image 2: 383479

Black pixels in image 2: 3810825

White pixels in image 3: 151132

Black pixels in image 3: 4043172

White pixels in image 4: 59426

Black pixels in image 4: 4134878

White pixels in image 5: 81014

Black pixels in image 5: 4113290

Percent white px:

/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_Sk658 Llobe ch0100
17.jpg:

0.6571054458618164% White | Depth: 45 microns

/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_Sk658 Llobe ch0100
30.jpg:

0.9011983871459961% White | Depth: 200 microns

/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_SK658 Slobe ch0100
98.jpg:

9.142851829528809% White | Depth: 10000 microns

/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_SK658 Slobe ch0101
15.jpg:

3.6032676696777344% White | Depth: 8800 microns

/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_SK658 Slobe ch0101
59.jpg:

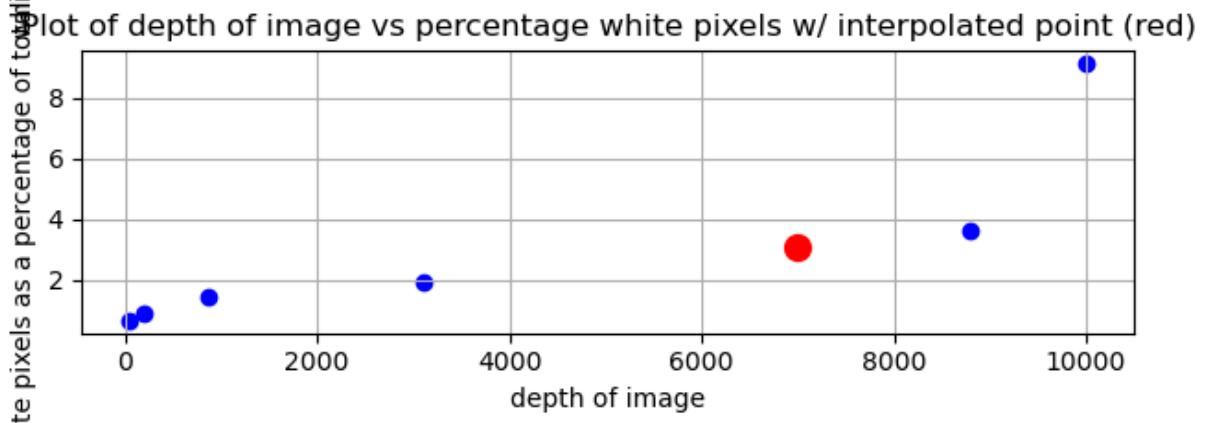
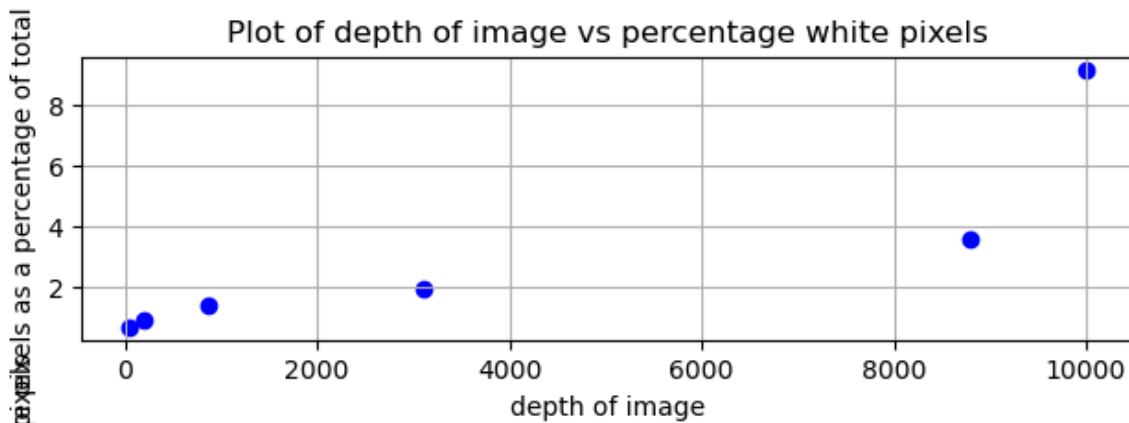
1.4168262481689453% White | Depth: 860 microns

/Users/LuckyStrawb3rry016/Desktop/Comp BME/Project 2/MASK_Sk658 Llobe ch0101
74.jpg:

1.9315242767333984% White | Depth: 3100 microns

CSV file 'Percent_White_Pixels.csv' has been created.

The interpolated point is at the x-coordinate 7000.0 and y-coordinate 3.075348703484786.



To validate our results and ensure a linear fit showed strong correlation with the data, we performed a linear regression with our linear fit. Our R² value of our linear regression was 0.77, indicating there is a strong correlation between depth of image and percentage of white pixels assessing with a linear relationship.

```
In [14]: '''Module 2 Verification and Validation: Linear Regression Analysis'''

from sklearn.linear_model import LinearRegression
from sklearn.metrics import r2_score
import numpy as np

# Convert lists to numpy arrays and reshape for sklearn
x = np.array(depths).reshape(-1, 1)
y = np.array(white_percents)

# Create and fit the linear regression model
model = LinearRegression()
model.fit(x, y)

# Predict y values based on the fitted line
y_pred = model.predict(x)

# Calculate the R-squared value
r2 = r2_score(y, y_pred)

# Print regression equation and R-squares
```

```

print(colored("Linear Regression Verification", "yellow"))
print(f"Equation: y = {model.coef_[0]:.4f}x + {model.intercept_: .4f}")
print(f"R2 value: {r2:.4f}")

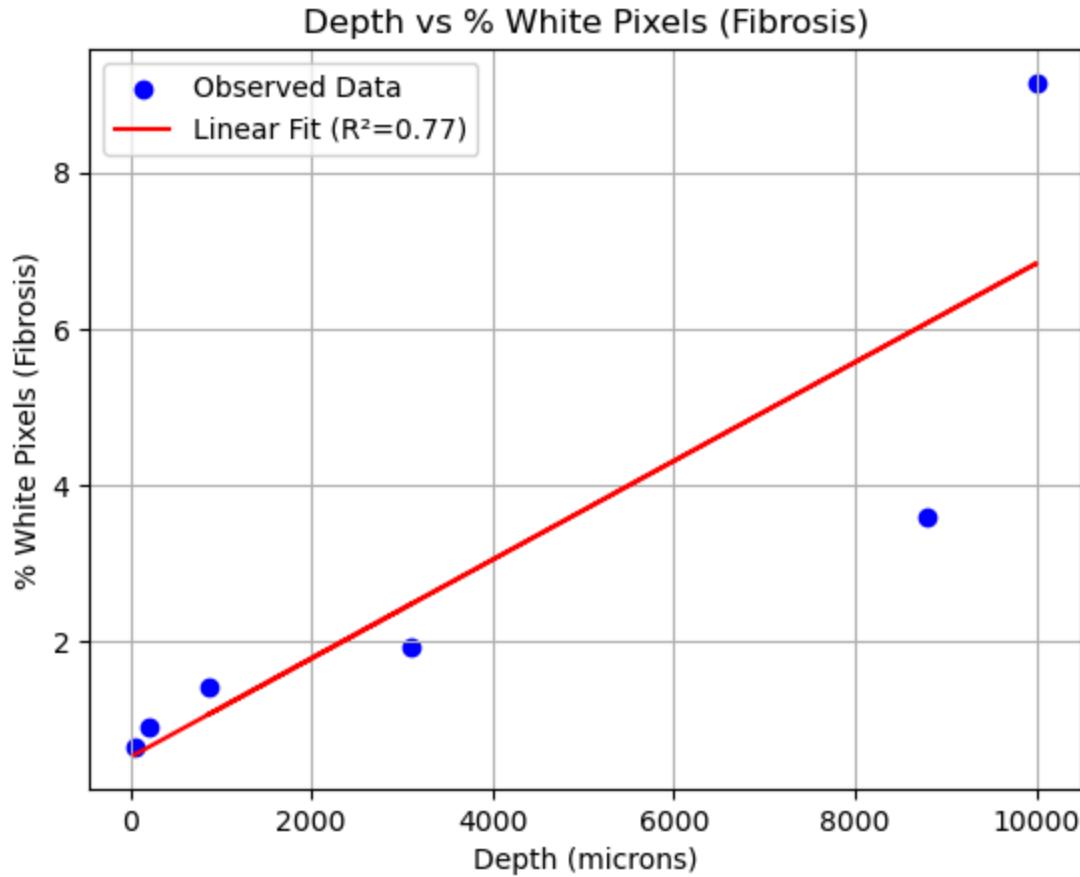
# Plot the data with regression line
plt.figure()
plt.scatter(x, y, color='blue', label='Observed Data')
plt.plot(x, y_pred, color='red', label=f'Linear Fit (R2={r2:.2f})')
plt.title('Depth vs % White Pixels (Fibrosis)')
plt.xlabel('Depth (microns)')
plt.ylabel('% White Pixels (Fibrosis)')
plt.legend()
plt.grid(True)
plt.show()

# Interpretation section
if model.coef_[0] > 0:
    trend = "increases"
else:
    trend = "decreases"

print(colored(f"Interpretation: The regression slope is {model.coef_[0]:.4f}

```

Linear Regression Verification
Equation: $y = 0.0006x + 0.5226$
 R^2 value: 0.7686



Interpretation: The regression slope is 0.0006, indicating that fibrosis (% white pixels) increases with depth. The R^2 value of 0.77 indicates there is a strong correlation between depth and percent of white pixels with a linear fit, so the relationship between depth and percent of white pixels is likely linear.

Verify and validate your analysis:

- Verification:
 - To verify that our analysis produced interpretable results and that our data were sensible, we examined both the raw data and the linear regression relationship between tissue depth (μm) and fibrosis extent (% white pixels).
 - We also ensured that we used multiple images from different depths and sections in the lung (not all from the same area), which showed a consistent increasing trend, confirming that a single outlier did not drive the relationship.
 - The regression analysis yielded a positive slope of 0.0006 and an R-squared value of 0.77, indicating a strong linear relationship between depth and fibrosis.
 - Therefore, this verifies that as tissue depth increases, the percentage of white pixels (representing collagen-dense fibrotic tissue) also increases.
 - This trend is biologically plausible, as in fibrotic lungs, deeper regions tend to experience lower ventilation and higher mechanical strain, which promotes extracellular matrix deposition and collagen accumulation.
 - The high R^2 value (close to 1) suggests that depth is related to the extent of fibrosis, and the extent increases linearly with depth.
- Validation:
 - To validate our findings, we compared our fibrosis trend (dependent on depth) to evidence from published literature on the distribution of lung fibrosis.

Several studies describes an accumulation of fibrosis in deeper lung regions which is consistent with our observations Barratt et al., American Journal of Respiratory and Critical Care Medicine, 2018

 - This study found that in idiopathic pulmonary fibrosis (IPF), fibrotic lesions begin in the subpleural and basal regions (edges of the lungs) and they then spread towards the lung interior (progression of the disease)
 - This pattern suggests that fibrosis tends to be more severe in deeper tissue layers, which aligns with our analysis, which found that the percentage of white pixels (representing fibrotic tissue) increased with increasing tissue depth.
- Overall, our analysis reveals that the extent of fibrosis increases with tissue depth, as confirmed by a statistically significant linear regression ($R^2= 0.77$) and supported by consistent findings in a relevant study. This suggests that our image-processing pipeline effectively reflects the biological trend of variation in fibrosis distribution within the lung.

Conclusions and Ethical Implications:

(Think about the answer your analysis generated, draw conclusions related to your overarching question, and discuss the ethical implications of your conclusions.

Conclusion: With the verification and validation of our results, we found strong correlation and a linear relationship between lung depth and percent of white pixels as a percent of total pixels. To explain, this means there is a strong correlation and a linear relationship between the depth of the lung and the extent of fibrosis.

Ethical Implications: Our model implies that fibrosis may not be uniformly distributed within the lung. The ethical implications of this data are related to wellbeing/future, such as a reduction in the number or extent of biopsy of tissue. Biopsy involves removing a sample of the tissue with minimally invasive surgery (more about this can be found in the background section). To take into consideration the depth of the lung, the extent of fibrosis can be predicted and even if a biopsy does not yield a definitive diagnosis, data collected from human trials of lung depth and fibrosis extent can be used to compare and extrapolate a potential measure of lung fibrosis, and reduce error in biopsy results.

Limitations and Future Work:

(Think about the answer your analysis generated, draw conclusions related to your overarching question, and discuss the ethical implications of your conclusions.

Limitations: Our data itself has a couple limitations. In our image slides, the blood vessels were traced out as white pixels, which increased the white pixel count. This can lead to errors and either strengthen or weaken our relationship between depth and lung fibrosis. To better understand the relationship, we would have to find a way to computationally remove these white boundaries. The other limitations are inherent with how the data is collected. We also only used 6 images at different depths, and lung fibrosis is more complex than that. We would have to increase the number of cross sections to get a better picture of what is happening. Considering that these images are cross sections of a rat's lungs with pulmonary fibrosis, this model may not exactly represent the extent of fibrosis in the human body. Also, the lung fibrosis is induced in the rat with Bleomycin, so the disease studied is no longer idiopathic, which could lead to potential limitations in the use of the data to model idiopathic disease.

Future Work: With this data and model, the first potential expansion would be to create a lung biopsy device. This device could take into consideration the depth from which the sample of tissue is retrieved and help predict the extent of fibrosis at this depth, leading into future research on progression by lung depth and maybe even potential treatments with extent of lung fibrosis and locality (depth) taken into consideration. This data could also be used in a similar way to create a more extensive learning approach rather than a simple linear relationship, such as incorporating depth-specific models to evaluate the

potential progression of the disease, maybe it is not as simple as we think. Machine learning models could take into account the depth and visual amounts of scar tissue/fibrosis coupled with patient statistics of lung function metrics (listed in the background section) to provide a more detailed progression of the disease and call for better treatment.

NOTES FROM YOUR TEAM:

This is where our team is taking notes and recording activity.

10/02/25- Began gathering background research, set up code to extract and graph pixel percentages by depth for 6 images.

10/07/25- Went over matrices and calculating regression with numpy, finished majority of background research.

10/07/25-10/15/25- Adjusted the code to broaden the depth range of the 6 images analyzed, verified data using linear regression, drew conclusions from data.

QUESTIONS FOR YOUR TA:

These are questions we have for our TA. [ANSWERED] We were just curious about the real world applications of this jupyter notebook, what are the research implications of knowing the pixel count by depths of lung fibrosis?

References:

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