

Al-Based Method for Quantification of Bleomycin-Induced Pulmonary Fibrosis in Mouse

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OBJECTIVE

To present an automated method for quantification and scoring of pulmonary fibrosis (PF) in mice, based on histological features like collagen deposition, lumen area, septal thickness, and inflammatory cells.

INTRODUCTION

- ▶ PF is a chronic progressive lung disease with poor prognosis and no effective therapy. Preclinical evaluation of novel therapeutic agents in an animal model of Bleomycin-induced lung fibrosis is critical in drug development.
- Modified Ashcroft's score [1],[2] is traditionally used for quantification of fibrosis in histological sections of the lung. However, manual and semi-automated scoring procedures on microphotographs are subjective, time-consuming and on a restricted field of view.
- ➤ We present a deep learning/image processing based method for automated quantification of PF on whole slide images (WSI). Performance of the method is compared with manual scoring by the pathologist.

MATERIALS

A) Specimen

Species & Study groups: Male C57BL/6 mice

Table 1: Details of study groups (20 animals/group)

Group	Inducing Agent (Intratracheal)	Treatment Regimen	
Sham	50µl Normal saline	D7 to D21	
Path (Vehicle control)	BLM* in 50µl Normal saline	D7 to D21	
Treated (Pirfenidone)	BLM in 50µl Normal saline	100mpk, PO, BID, D7 to D21	

*BLM: Bleomycin

Tissue

Two FFPE (Formalin fixed paraffin embedded) sections of lungs, 4-5µm in thickness stained with Picrosirius red (PSR) and Hematoxylin & Eosin (H&E) staining, for each animal.

B) Image Acquisition

Scanning at 40x magnification using Hamamatsu Nanozoomer XR scanner.

METHODS

A) Training, Testing and Fine Tuning

- ➤ WSI selection 20 WSI were selected from three study groups and divided into 512x512 tiles at four magnifications (40x, 10x, 2.5x, and 1x).
- Segmentation performed using:
- Image processing techniques for collagen deposition (40x) and luminal area (2.5x) on PSR stained images.
- Deep learning model (ResNet) for inflammatory cells and septal regions (thick & thin) at 40x magnification on H&E stained images.

The process flow for quantification of pulmonary fibrosis is depicted below:

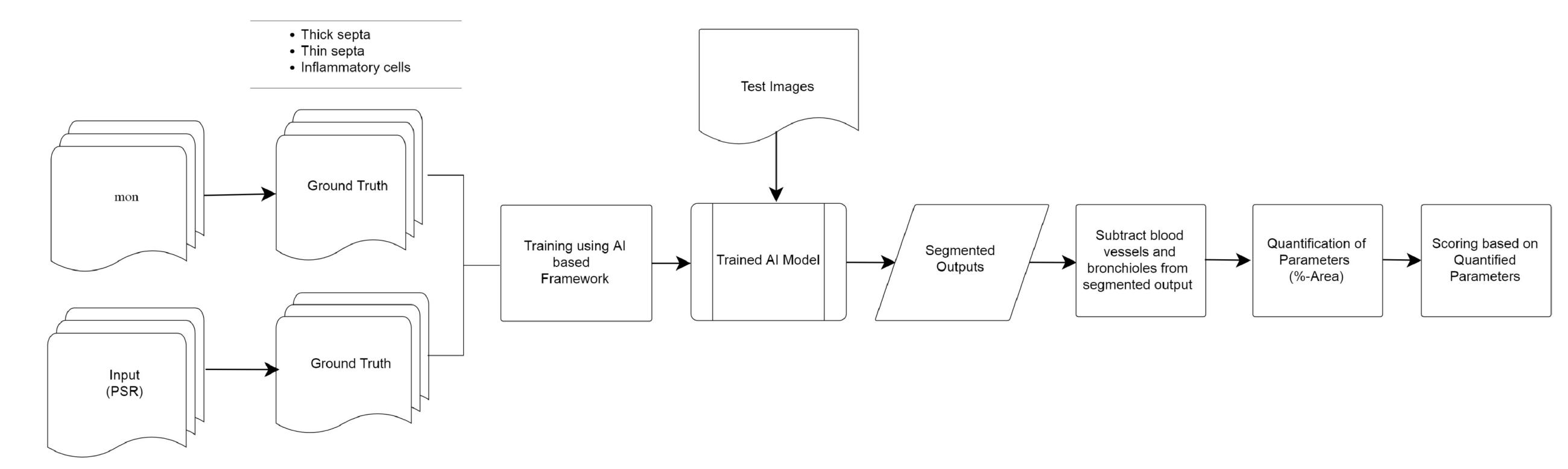


Figure 1: Process flow for quantification of pulmonary fibrosis

- Major blood vessels and bronchioles were excluded from quantification.
- Parameters were quantified based on total percentage area of lung parenchyma.

B) Validation

- 40 WSI from Path (15), Sham (14) and Treated (11) groups were analyzed and scored by the algorithm.
- The scores were compared with the modified Aschroft's scores generated through manual evaluation by the pathologist.

Image 1:

H&E stained 512x512 tile - mouse lung at 40x magnification (Path group)

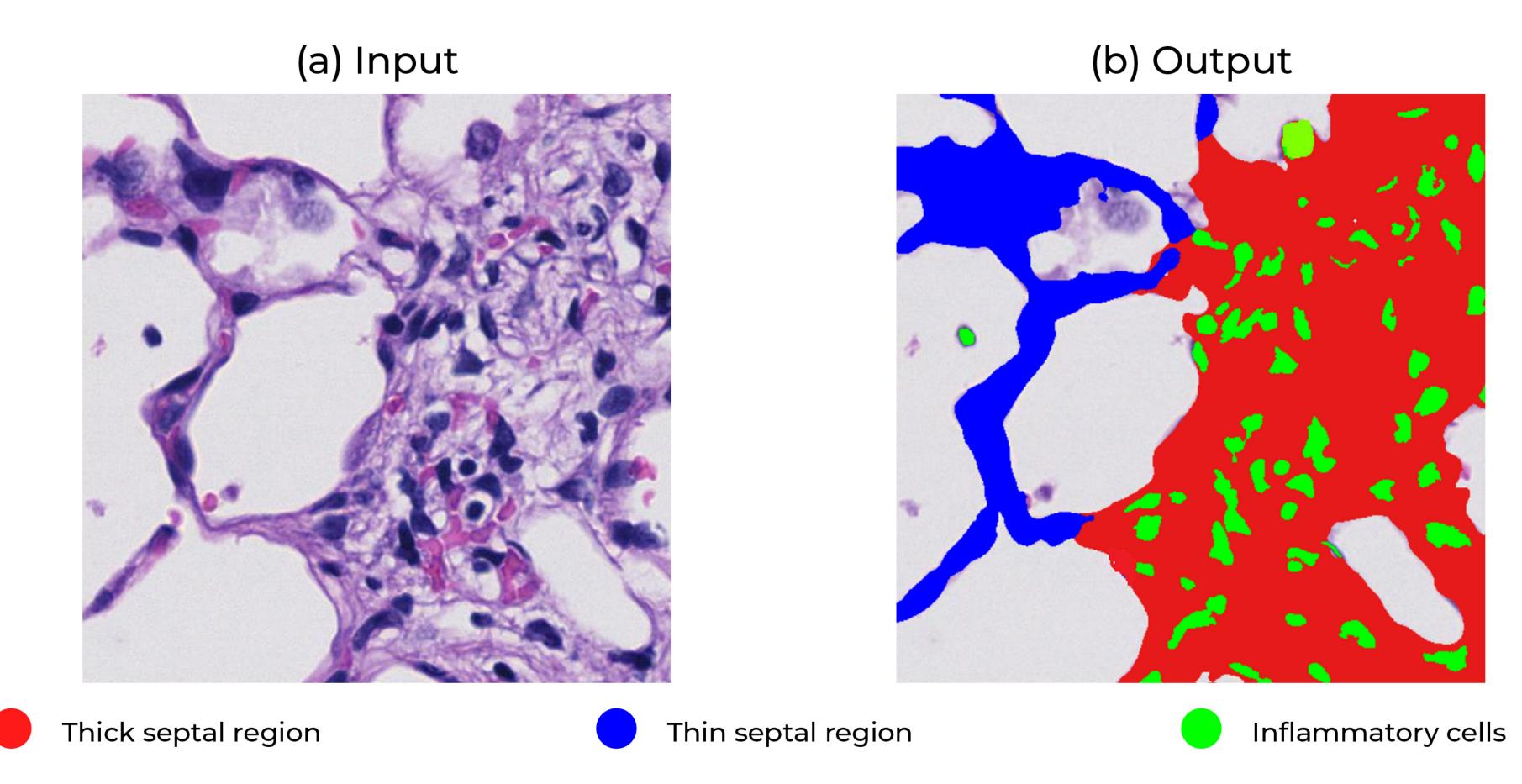
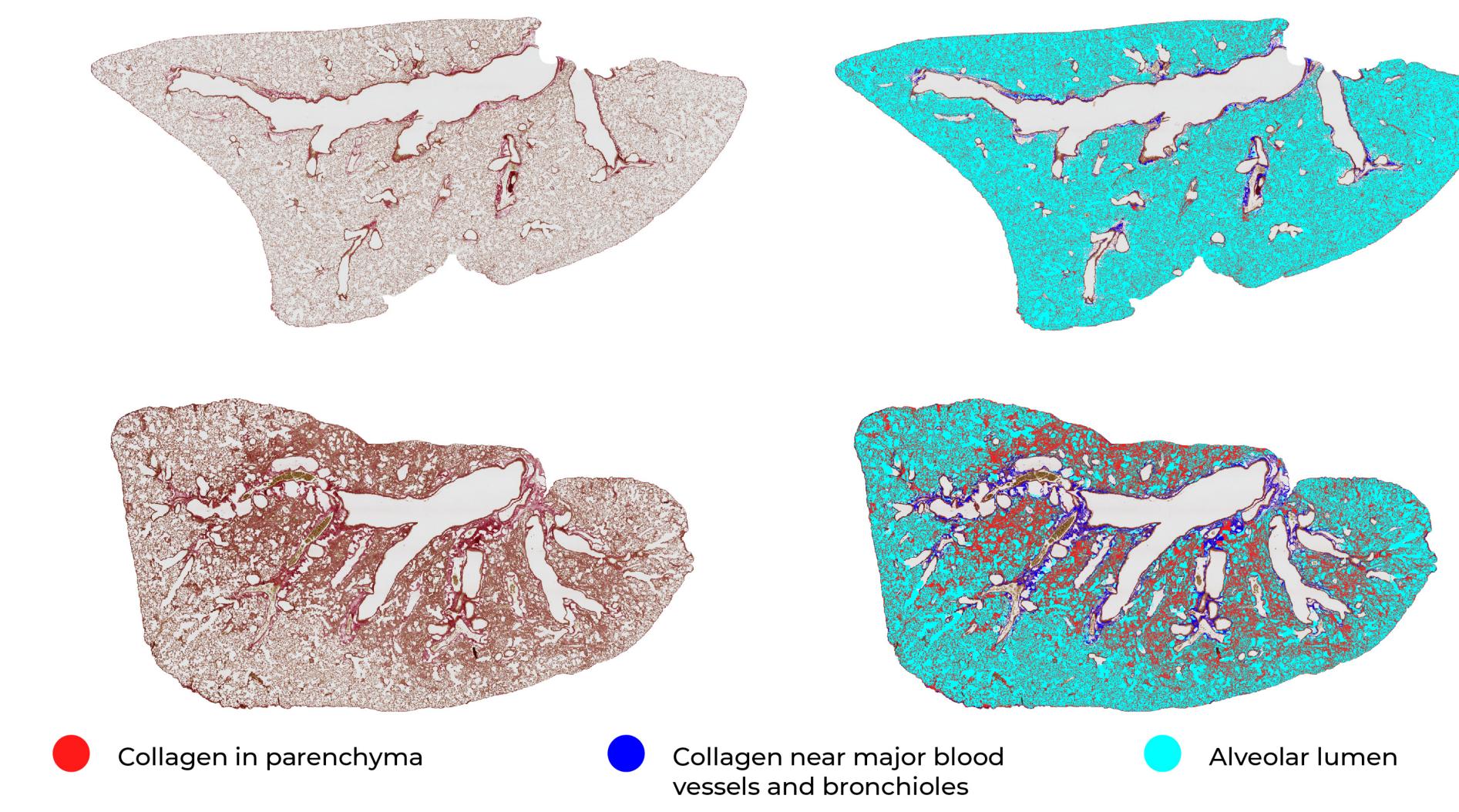


Image 2:

PSR stained whole slide image - mouse lung at 2.5x magnification (Sham group)

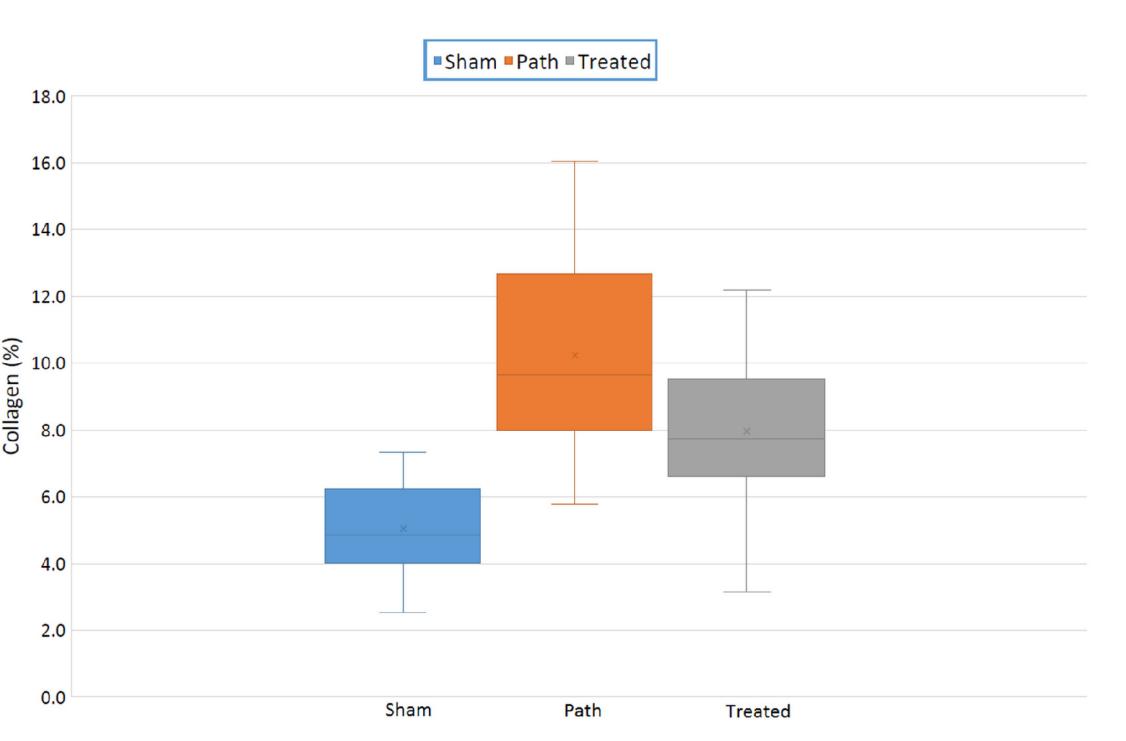
Image 3:PSR stained v

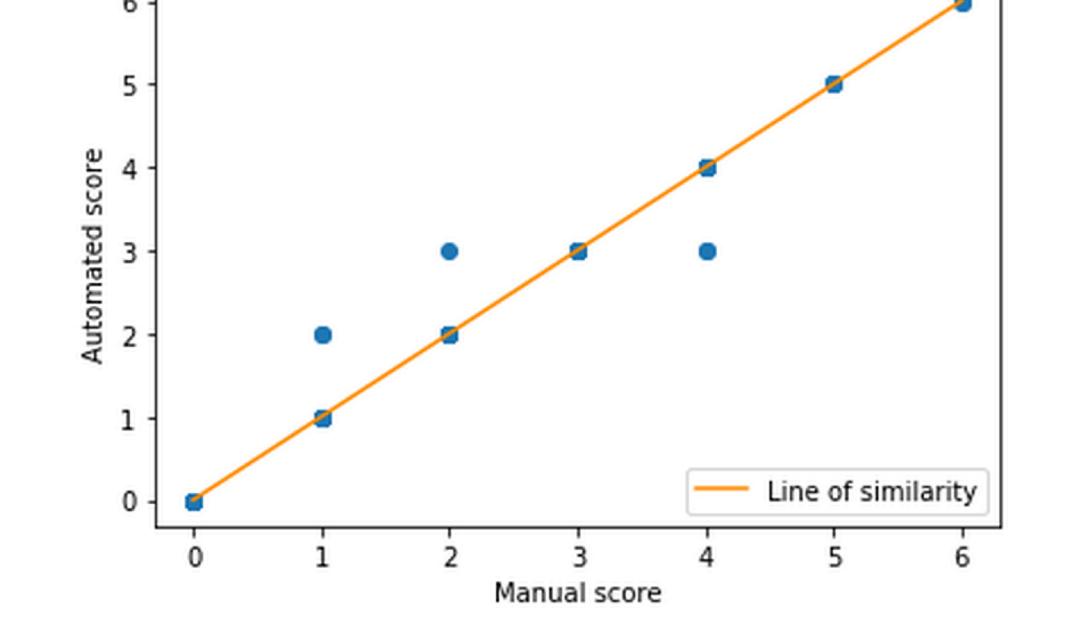
PSR stained whole slide image - mouse lung at 2.5x magnification (Path group)



RESULTS

- Dice accuracy of 98.76% was achieved for segmentation, averaged over all four histomorphometric parameters.
- Performance characteristics of the algorithm in comparison with manual scoring by the pathologist showed overall accuracy and precision of 98.5% and 92.8% respectively.





Validation with Pathologist score

Figure 2: Collagen area computed by algorithm across Path, Sham and Treated groups

Figure 3: Comparison between manual and automated modified Ashcroft's score

Table 2: Performance characteristics of the algorithm in comparison with manual scoring by the pathologist

Class	Specificity	Sensitivity	Precision	Accuracy
0	1.000	1.000	1.000	1.000
1	0.967	1.000	0.818	1.000
2	0.967	0.875	0.875	0.981
3	0.950	0.800	1.000	0.938
4	0.950	0.889	0.800	0.980
5	1.000	1.000	1.000	1.000
6	1.000	1.000	1.000	1.000
Avg			0.928	0.985

Note: Classes 7 and 8 were not observed.

CONCLUSION

The automated algorithm quantified and scored pulmonary fibrosis in WSI of mice lungs with high accuracy and precision. Comparative performance analysis of scoring by the algorithm showed concordance with modified Aschroft's scoring performed manually by the pathologist.

This automated algorithm can hence aid the pathologist in expeditious, accurate and reproducible pulmonary fibrosis scoring in WSI of the lung.

REFERENCES

- 1] Ashcroft, T., Judy M. Simpson, and V. Timbrell. "Simple method of estimating severity of pulmonary fibrosis on a numerical scale." Journal of clinical pathology 41.4 (1988): 467-470.
- [2] Hübner, Ralf-Harto, et al. "Standardized quantification of pulmonary fibrosis in histological samples." Biotechniques 44.4 (2008): 507-517.