Quantitative Analysis of Microglia IHC in Fibrotic Mouse Brains

Author
Stella Schultz (s.m.schultz@student.tudelft.nl)

Supervisors
Héctor Tejeda Mora, Hélène F. E. Gleitz

Responsible Professor
Michael Weinmann

How can microglia burden in whole-brain Iba1-stained sections be quantified using an automated, tile-based image analysis/machine learning pipeline?

Introduction

Myeloproliferative neoplasms (MPNs) are types of chronic blood cancers characterized by fibrosis. In 2021 there were almost 350,000 cases globally [1]. The connection between MPNs and microglia in the brain has not been explored, and understanding this may lead to more effective treatment for patients.

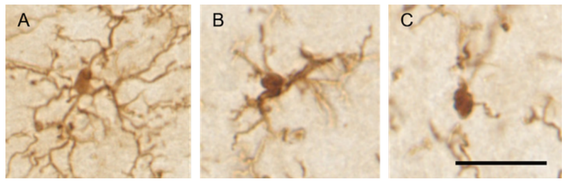

Figure 1: Examples of microglia morphological states. Scale bar 25µm. A: Homeostatic B: Reactive C: Amoeboid

To quantify fibrosis we analyze microglia morphological states. The states of interest are homeostatic (healthy), reactive (reacting to stimulus), and amoeboid (neuro-inflammation) and differ in the level of branching, with homeostatic having the most extensive branching and amoeboid the least.

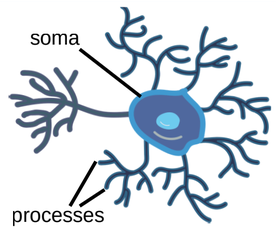

Figure 5: Microglia diagram

Methodology

Cell Localization

Soma localization consisted of a u-net machine learning model to create cell soma heatmaps. Processes were localized by binarizing the image and using a watershed algorithm seeded at the localized soma points.

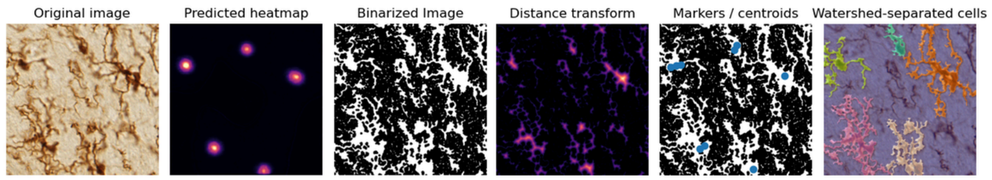

Figure 2: Example of full localization pipeline steps from input image to localized cells.

Cell Analysis

Cell features were extracted with Sholl analysis and skeletonization. Convolution was used to calculate the number of terminal points for each cell.
Brain slices were annotated by region and within each region Gaussian mixture models (GMM) clustered cells based on the calculated features.

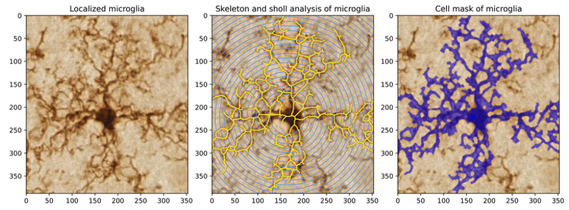

Figure 3: Microglia morphological feature extraction example.

Results

- Statistically significant difference in overall cell density between control and fibrotic brains
- Statistically significant difference in cell density in cerebral cortex between control and fibrotic brains
- No significant difference based on calculated features and clustering

Conclusion

Fibrosis impacts the brain as evidenced by the decrease in microglia. Quantifying this impact using these features was ineffective and may require stricter classification rules or shifting focus to analyzing factors beyond cell morphology such as their transcriptome.

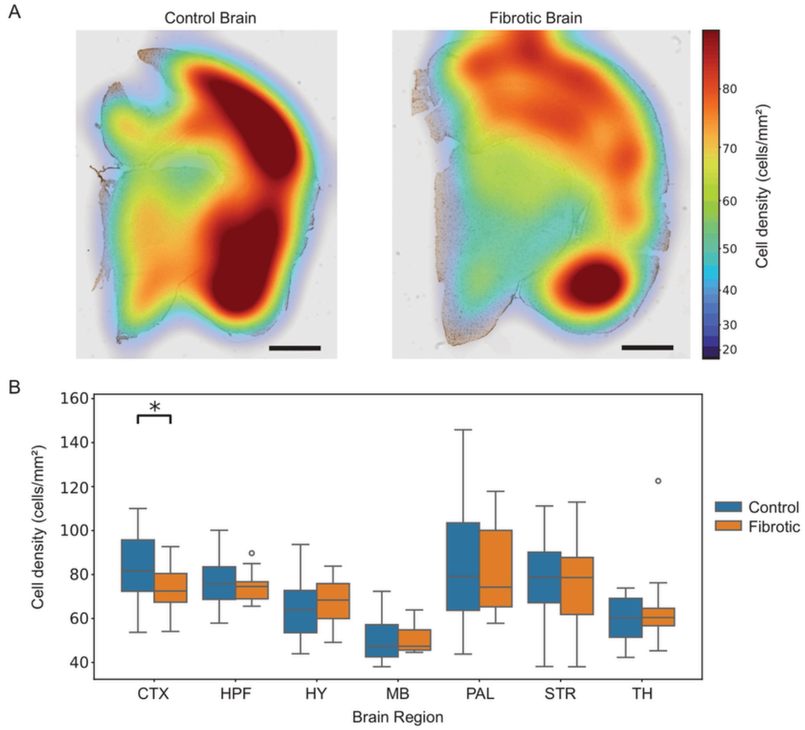

*Figure 4: A: Cell density heatmap in sample control and fibrotic brain slices. Scale bar: 1.25mm B: Cell density in control and fibrotic brain slices grouped by brain region. Statistically significant results denoted by *.*

Limitations & Future Work

- Cell localization is not perfect due to differences in focus, overlapping microglia, and overlapping tissue. Future work can improve upon localization specificity and sensitivity.
- Images analyzed were 2D, future work should investigate 3D images for a more complete cell representation
- A promising future direction is using supervised learning to extract features and classify cells into the morphological states rather than soft cluster assignments

References:

[1] X. Gou, Z. Chen, and Y. Shangguan, "Global, regional, and national burden of myelodysplastic syndromes and myeloproliferative neoplasms, 1990-2021: an analysis from the global burden of disease study 2021," Front Oncol, vol. 15, p. 1559382, Mar. 2025, doi: 10.3389/fonc.2025.1559382.