Automated Image Analysis of Neuroinflammation: staging microglia activation in the brain

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Introduction

Microglia play a key role in brain inflammation, transitioning between homeostatic, pro-inflammatory, and anti-inflammatory states. While previous have studies focused on distinct phenotypes, this work highlights intermediate states in both morphology and metabolism. Using fluorescent 2D/3D imaging and lifetime measurements, our pipeline enables accurate binary and multiclass classification. Notably, we introduce multiclass classification and an automatic clustering approach, defining a continuous activation scale based on cell morphology.

Materials and Methods

The *in vivo* acute viral model of LRRK2 Parkinson's disease was used, overexpressing human wildtype (WT) or G2019S LRRK2 (helper-dependent adenovirus, hdAd5) in the striatum and nigra post injection. The mice brains were retrieved 3 weeks after the injection, cut into slices of 30μm and stained with rabbit anti-Iba1 (primary antibody) and anti-rabbit 488 (secondary antibody). The slices were viewed using a Leica TCS SP8 FALCON Multiphoton microscope with which 3D cell images were acquired using a x63 lens together with the respective lifetime data measured after 20 seconds of photon collection from the sample excited with a wavelength of 740nm. [1] The complete dataset of 3D single-cell images and respective mean NAD(P)H lifetime values of the cell bodies, includes 1082 microglial cells. Additional to the 3D images, a 2D projection image was produced for each cell of the dataset. Depending on their lifetime value and morphology, the cells were attributed one of two labels, corresponding to the absolute activation/non-activation states.

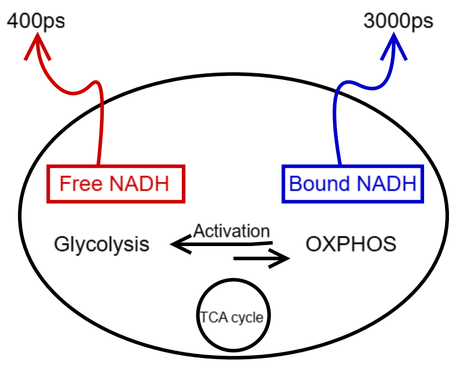


Figure 1:NADH states and respective Lifetimes

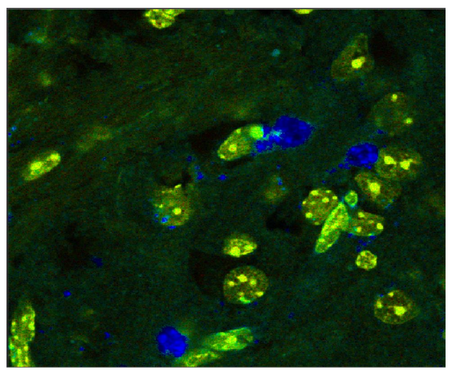


Figure 2:"Blue" color coded pixels have lower lifetime

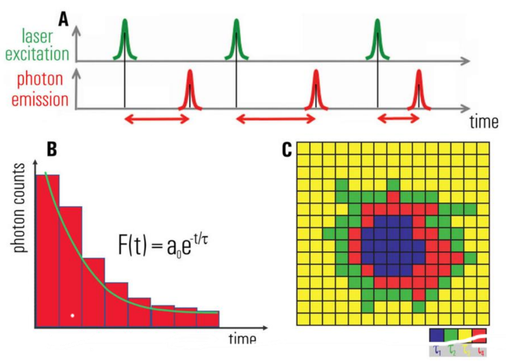


Figure 3:Time-Correlated Single Photon Counting

Geometrical features of cell body area, circularity, aspect ratio and eccentricity as well as branch length and branch thickness, were calculated from the 2D projection images and their analogous features from the 3D images datasets. Additionally, a 2D and 3D Convolutional Neural Networks were used to extract additional features from the two datasets respectively. To perform clustering the k-means algorithm was used, while for both the binary and multiclass classification using both morphological features and lifetime values was performed with the use of various classifiers including the Naïve Bayes, K-Nearest Neighbor, Logistic Regression, Random Forest, Decision Tree, Multi-layer Perceptron and a Supporting Vector Machine. Finally, for the continuous activation percentage prediction value, a CNN was trained on the cells' morphology alone.

Results

0. Microglia Characterization through FLIM to assess LRRK2 role in neuroinflammation

Before introducing machine learning algorithms, labeling of individual microglia cells was performed to examine neuroinflammation in both the striatum and nigra. Both the striatum and substantia nigra had increase of microglia activation compared to its wildtype in mice injected with the G2019S LRRK2 mutation (Fig.4). This aligns with the known role of LRRK2 in promoting inflammation through heightened kinase activity [2]. The substantia nigra tended to show more activated microglia overall, which could be due to its greater vulnerability to inflammatory stress and neurodegeneration in Parkinson's disease. The increase of activation shows that G2019S mutation shifts microglia into pro-inflammatory state.

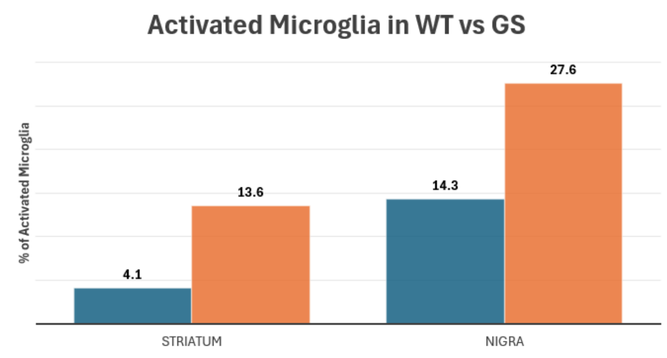


Figure 4

A. Clustering

The k-means algorithm grouped the cells based on their NADH lifetime value alone into four subgroups. Analysis has demonstrated the continuous nature of lifetime, reflecting the continuity of the metabolic transition from homeostasis to activated state (Fig.5). Clustering was also performed using morphological and CNN extracted features from the 2D-projection images resulting in four different categories with morphologies corresponding to the ones known in literature (Fig.6).

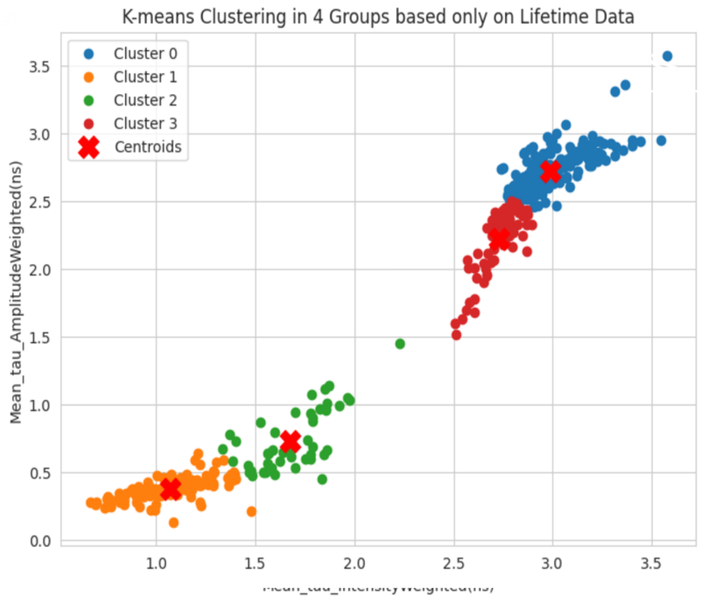


Figure 5

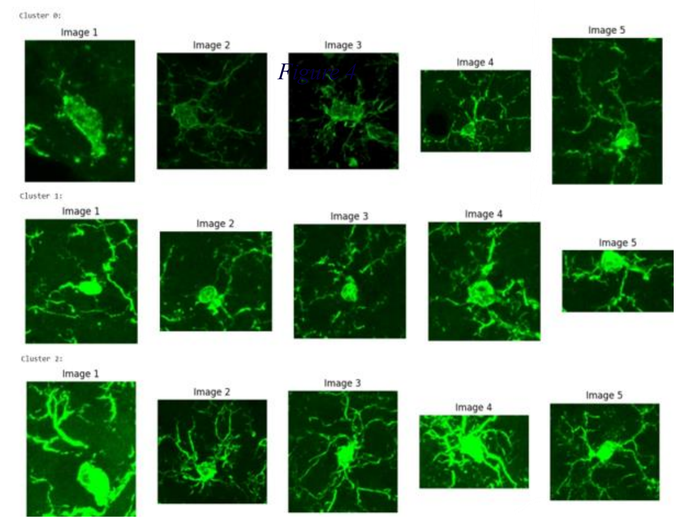


Figure 6

B. Classification

Highest classification accuracies were higher when trained on the 2D images geometrical features (Fig.7), while performance in both multiclass and binary classification was enhanced when NADH lifetime value was incorporated in the feature vector (Fig.8). As far as binary classification is concerned higher accuracy was achieved when training the classifiers with features extracted using a 3D CNN. Additionally, binary classification using lifetime values alone had very high accuracies as well for all models (Fig.9)

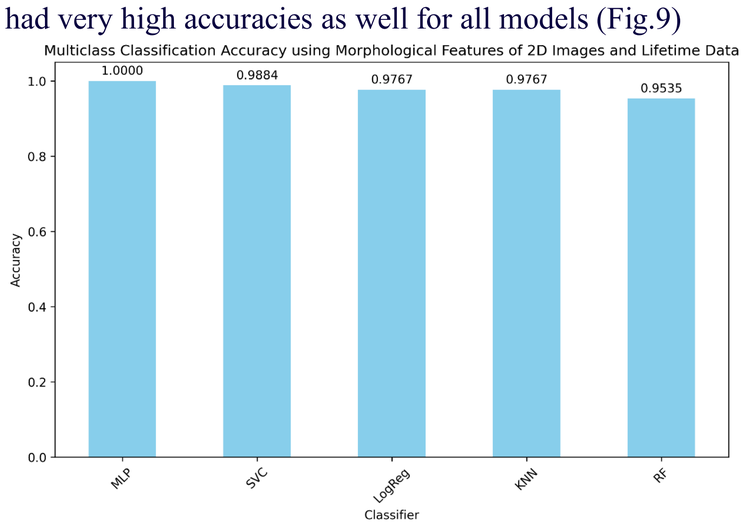
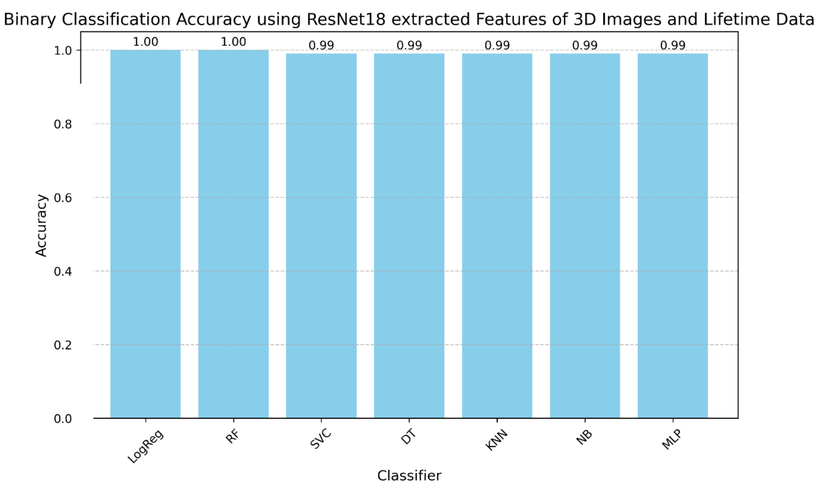


Figure 7



`Figure 8

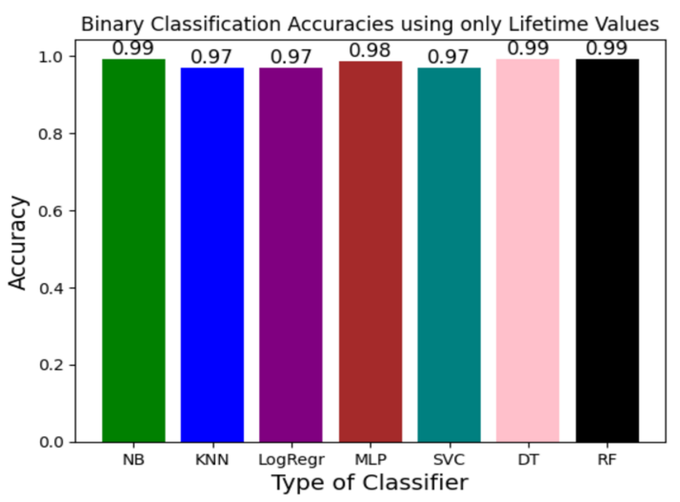


Figure 9

C. Regression

A continuous activation scale was introduced, to attribute to the microglia a percentage of activation based on their 2D morphology (Fig.10).

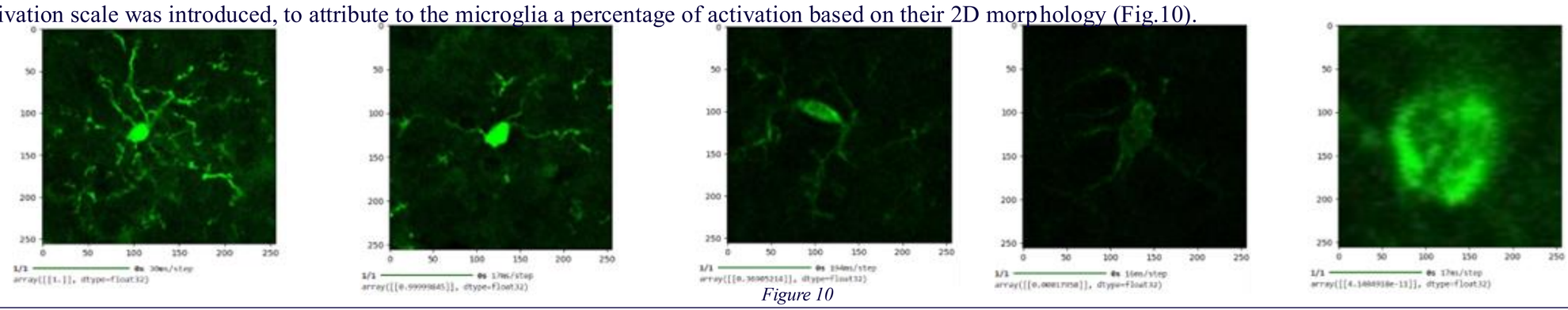


Figure 10

Conclusion

This work examines intermediate microglial metabolic states with the use of cell morphology and NADH lifetime values by proposing a continuous activation scale. It is also the first work to explore microglia clustering and classification using 3D images and the first work to perform multiclass microglia classification with the additional use of lifetime.

References

[1] Cao R, Wallrabe H, Periasamy A. Multiphoton FLIM imaging of NAD(P)H and FAD with one excitation wavelength. J Biomed Opt. 2020 Jan;25(1):1-16.
[2] Mutti, V., Carini, G., Filippini, A., Castrezzati, S., Giugno, L., Gennarelli, M., & Russo, I. (2023). LRRK2 Kinase Inhibition Attenuates Neuroinflammation and Cytotoxicity in Animal Models of Alzheimer's and Parkinson's Disease-Related Neuroinflammation. *Cells*, *12*(13), 1799.