Computational profiling of astrocytes' activation patterns after mild fluid percussion injury

Jahandar Jahanipour[1], Xiaoyang Li[1], Hengyang Lu[1], John Redell[2], Pramod Dash[2], Dragan Maric[3], Badrinath Roysam[1]

[1]Electrical & Computer Engineering, University of Houston, Houston, TX

[2] Department of Neurobiology and Anatomy University of Texas Health Science Center at Houston, Houston, TX

[3] Flow Cytometry Core Facility, NINDS, NIH, Bethesda, MD

INTRODUCTION

Development of novel image analysis methods and pattern recognition techniques can provide powerful new tools to efficiently and accurately investigating large image datasets, and mine the resultant data to help identify altered signaling pathways and/or vulnerable cell populations that could be targeted for interventional treatment. Deep learning has been widely used in detection, segmentation and analysis of large image datasets. In this project, we define precise task for the machine to extract and learn abstract representations of the samples. Using these abstract representations, the software can then implement hierarchical clustering approaches to identify and profile heterogeneous responses among the different types of brain cells after an injury. These observations can help identify vulnerable regions and cell types within the brain, the distribution of cell types and cell status altered after injury, and provide clues to guide interventional strategies. In this study a scattering network (ScatNet) is used to extract the deep translation invariant features of each cell. These deep features are used in a hierarchical clustering algorithm to find similar groups of cells. We have designed a Graphical User Interface (GUI) to visualize the clusters on top of the images to give the biologists the ability to do further analysis. We report clustering of astrocytes based on basal morphology and texture features to profile their activation state after mild fluid percussion injury using immunostaining for markers related to cytoarchitecture, proliferation and intercellular signaling functions.

OVERVIEW

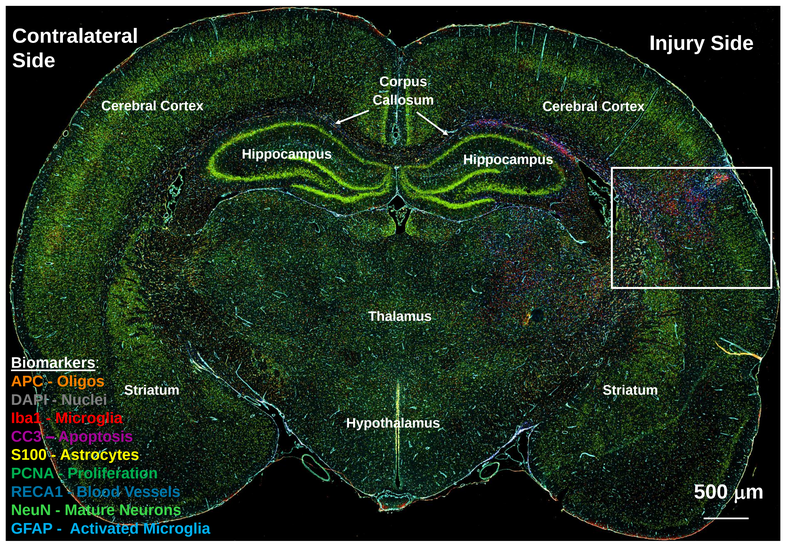


Figure 1. Highly multiplexed fluorescence immunohistochemistry image illustrating the complex cellular responses and tissue remodeling trigged by a mild traumatic brain injury (lateral fluid percussion injury, 1.5 atm, 14 d)

Neuronal Cell Classification	Biomarkers for neuronal phenotyping			
	NeuN	GAD67	Parvalbumin	Calretenin
GABAergic Neurons	Subset (+)	All (+)	Subset (+)	Subset (+)
Non-GABAergic Neurons	All (+)	All (-)	Subset (+)	Subset (+)
Astrocyte Classification	**Biomarkers for astrocyte phenotyping**			
	S100	APC	GFAP	GLAST
Resting Astrocytes	All (+)	Subset (+)	Subset (low)	Subset (+)
Reactive Astrocytes	All (+)	Subset (+)	All (high)	All (+)
Oligodendrocyte Classification	**Biomarkers for oligodendrocyte phenotyping**			
	S100	APC	MBP	PLP
Myelinating Oligodendrocytes	All (-)	All (+)	All (+)	All (+)
Non-myelinating Oligodendrocytes	All (-)	All (+)	All (-)	All (-)
Microglia Classification	**Biomarkers for microglia phenotyping**			
	S100	APC	Iba1	Tomato Lectin
Resting Microglia	All (-)	All (-)	All (+)	All (low)
Reactive Microglia	All (-)	All (-)	All (+)	All (high)
Phagocytic Microglia*	All (-)	All (-)	All (+)*	All (high)
Blood Vessel Classification	**Biomarkers for endothelial cell phenotyping**			
	S100	APC	RECA	Tomato Lectin
Endothelial Cells	All (-)	All (-)	All (+)	Subset (+)

Table 1. Boolean logic table for cell type classification

METHOD

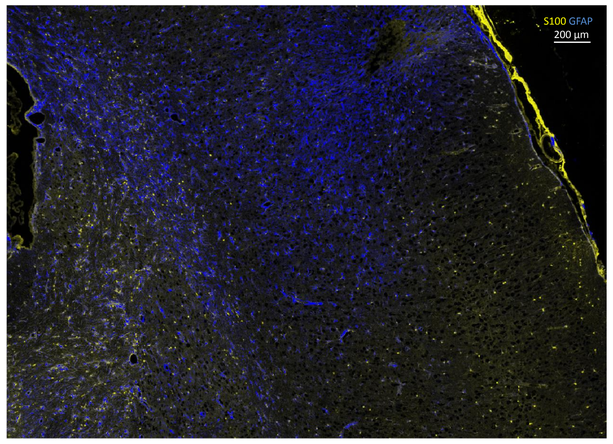


Figure 2. S100β and GFAP immunostaining from the selected region of interest shown in Figure1.

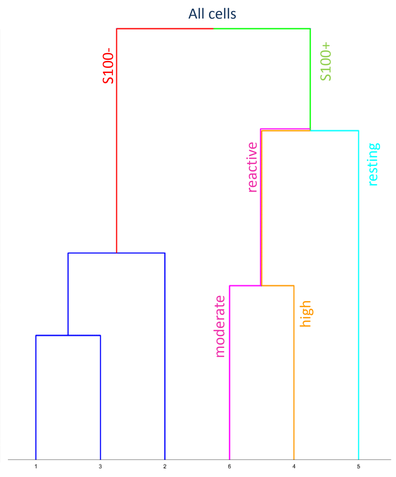


Figure 3. Dendrogram representing different subdivisions of cells after hierarchical clustering for activated astrocyte profiles

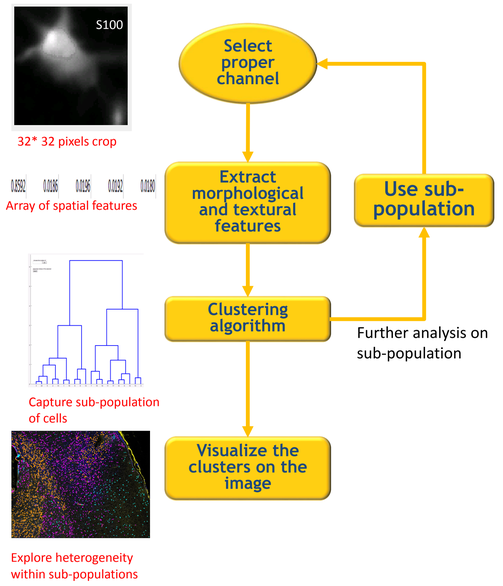
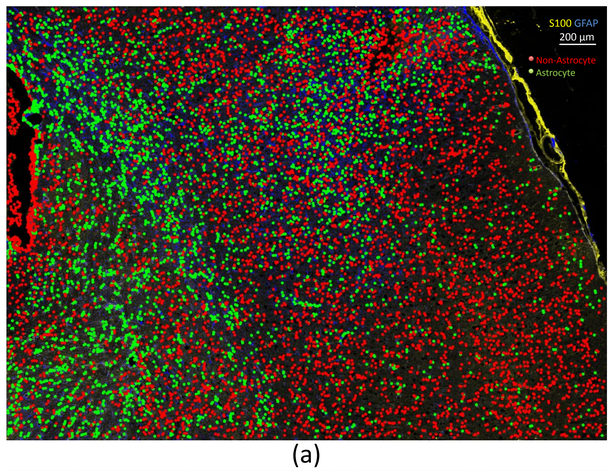
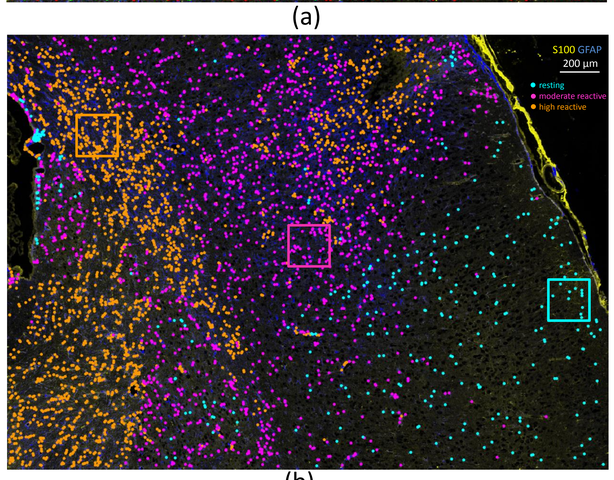


Figure 4. Image analysis pipeline

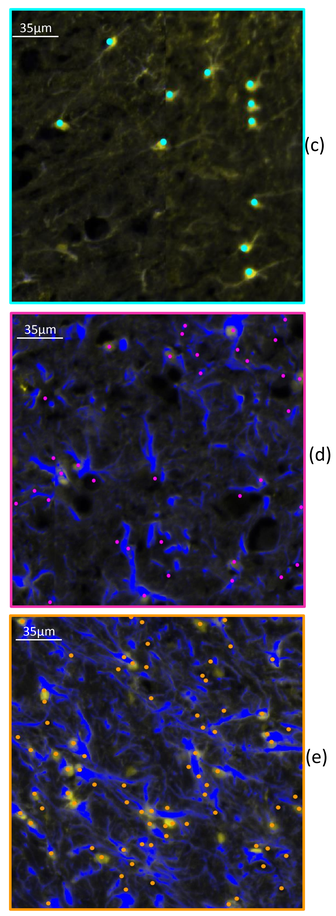
RESULTS



(a)



(b)



(c) (d) (e)

Figure 5. (a) clustering results of detecting astrocytes among all the cells using S100 and GFAP channels (b) clustering results of profiling activations of astrocytes using GFAP and GLAST channels – (c-e) close-up of boxes

Astrocyte detection	
Detected cell profiles	9924
Astrocyte	3998
Non-Astrocyte	5926

Table 2. Cell profile counts of detected cells classified into astrocyte and non-astrocyte populations based on S100 and GFAP immunostaining

Astrocyte activation profiles	
All astrocytes	3998
Resting	318
Moderate reactive	1595
High reactive	2085

Table 3. Cell profile counts of activated astrocytes in the RIO based on GFAP and GLAST immunostaining

CONCLUSIONS

- We are able to capture the spatial features of each cell, such as basal morphology and texture using defined sets of antibodies to identify cell-type and activation state.
- Using specific input channels, we can profile sub populations of cell types based on heterogeneity among the extracted spatial features.
- Analysis revealed a graded astrocyte response with spatial and regional features after a mild TBI.

REFERENCES

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