

OVERVIEW

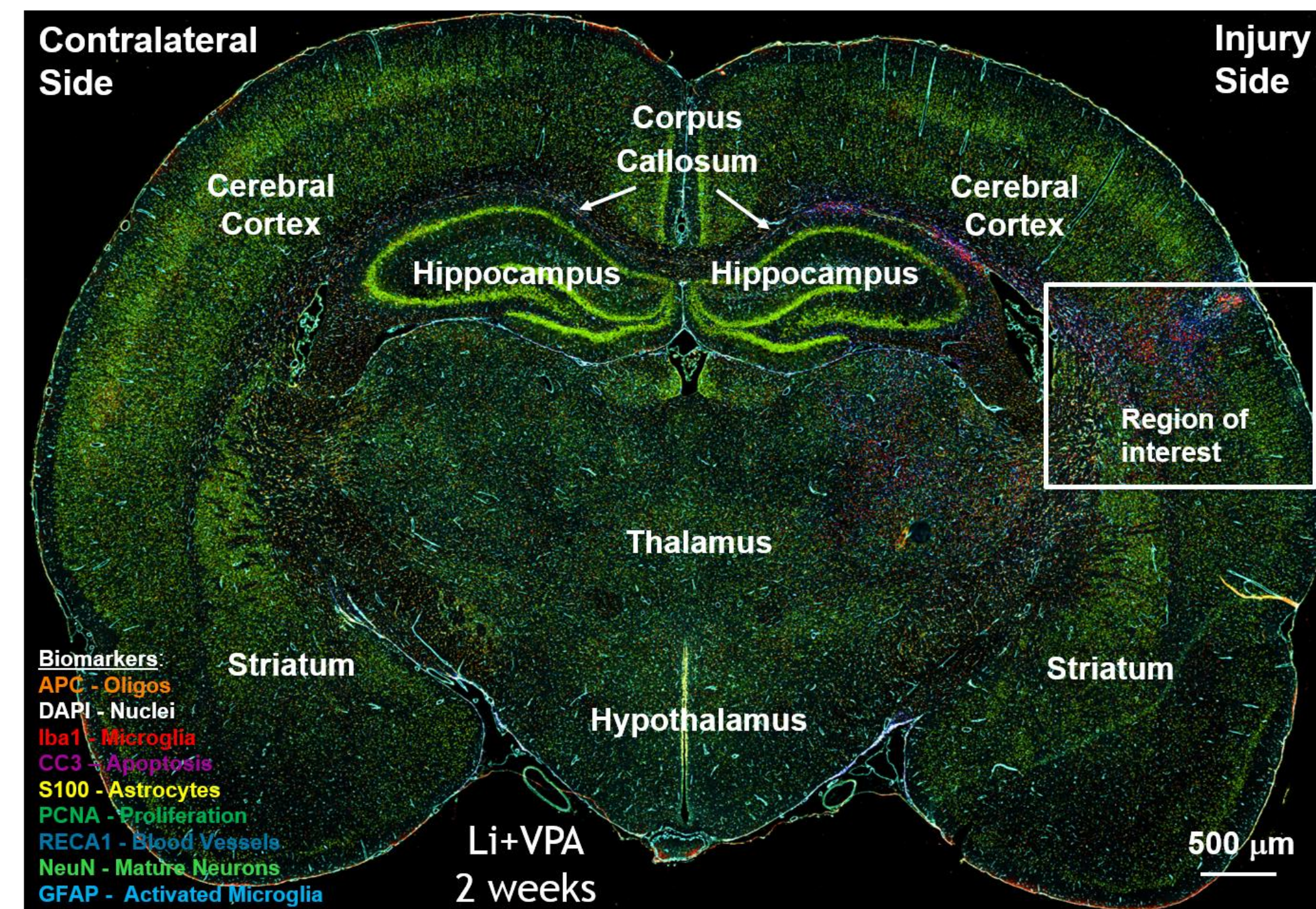


Figure 1. Highly multiplexed fluorescence immunohistochemistry image illustrating the complex cellular responses and tissue remodeling triggered by a mild traumatic brain injury (lateral fluid percussion injury, 1.5 atm, 14 days).

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.

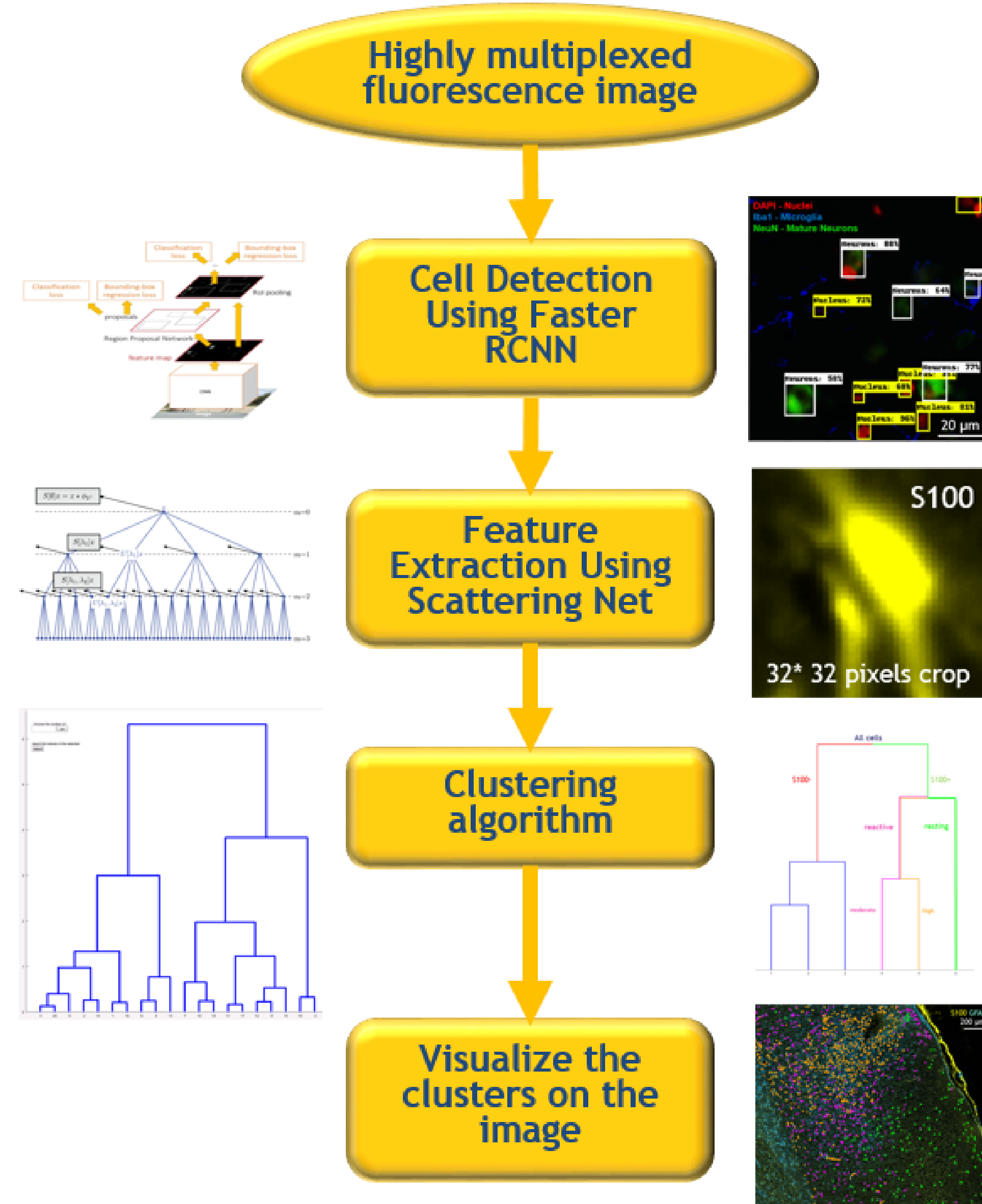


Figure 2. Image analysis pipeline.

FEATURE EXTRACTION

Conventional Cytometric Features:

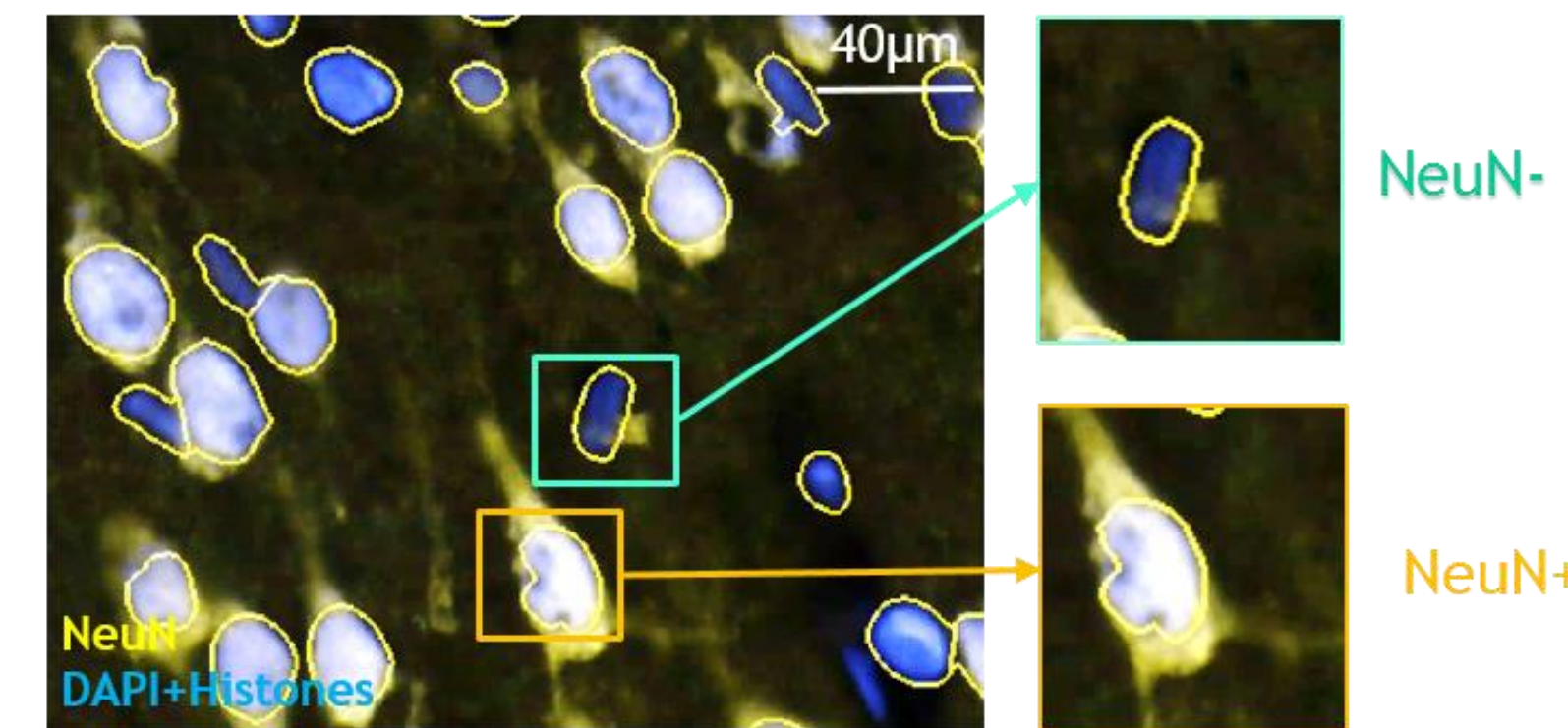


Figure 3. Nuclear segmentation of cells using DAPI +Histone channels.

Traditional Features:

- ✗ **Nuclear morphological features** are not able to capture thorough molecular signature.
- ✗ **Associative features** are dependent on nuclear segmentation of object.

Deep Features:

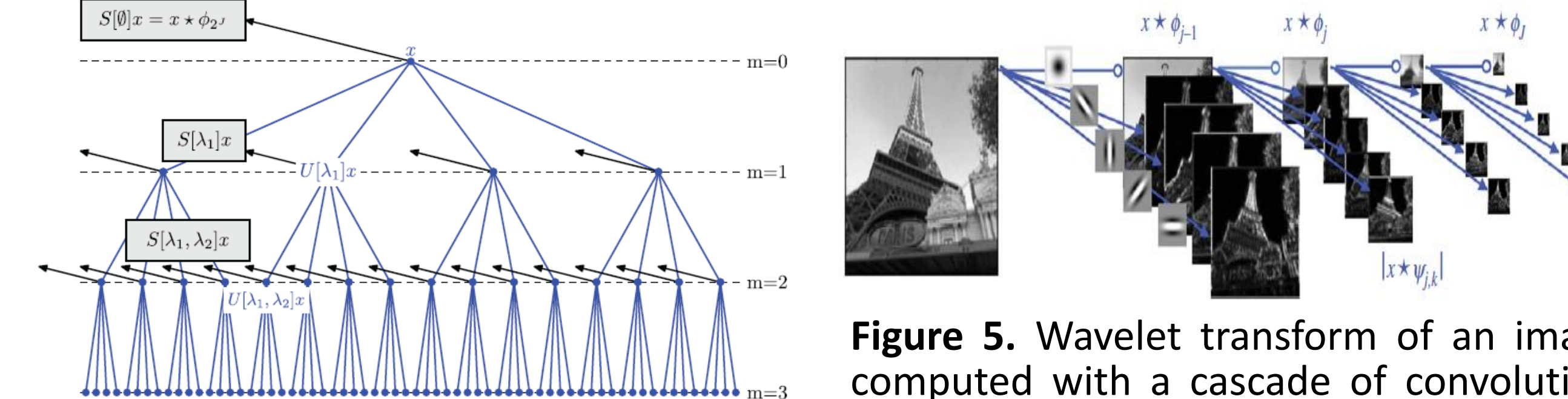


Figure 4. Scattering network formed by wavelet-modulus cascading.

1. Decomposing the signal in a family of wavelets with all dilation and rotation:

$$Wx(t) = \underbrace{\{x * \phi(t)\}}_{\text{average}}, \underbrace{\{x * \psi_\lambda(t)\}}_{\text{Convolution of signal with wavelets}}_\lambda$$

2. Iteratively repeating the operator W .

build multi layers → deep network

- ✓ Features are **invariant to translation** and **stable to deformation**.
- ✓ It does not require **extensive labeling** and **network training** in order to compute the deep features.
- ✓ It uses a multiscale set of **pre-defined** wavelet based filters.
- ✓ Features have the advantage of **mathematical rigor** unlike trained convolutional networks.

Deep features capture basal cell morphology and molecular distribution, JOINTLY.

RESULTS

1. Layered Cytoarchitecture of Cortical Brain Tissue

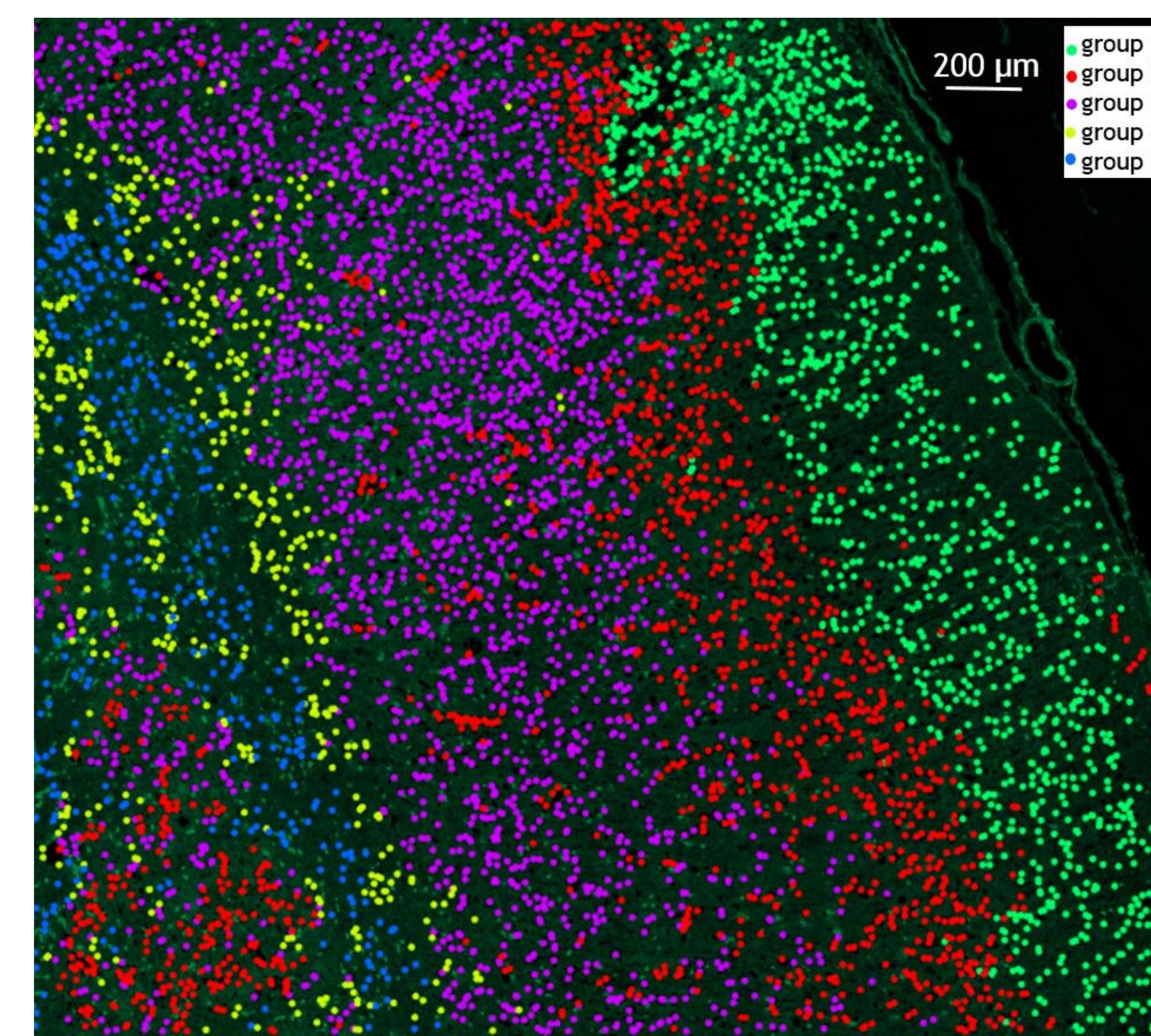


Figure 6. Clustering results of cells using APC/S100/PLP/MBP channels

IMPACT: Successfully recapitulated the layered cytoarchitecture of cortical brain tissue in an unsupervised manner based on oligo-glial features alone.

2. Unsupervised Cell Type Cluster Labeling

Actual	Predicted	
	IBA1 -	IBA1 +
-	8,832	260
+	268	564

Overall Accuracy = 94.68%

Actual	Predicted	
	S100 -	S100 +
-	7,693	137
+	731	1,273

Overall Accuracy = 91.17%

IMPACT: Provided an unsupervised alternative approach for cell type identification with > 90% accuracy.

3. Deep Profiling of Astrocyte Activation Status

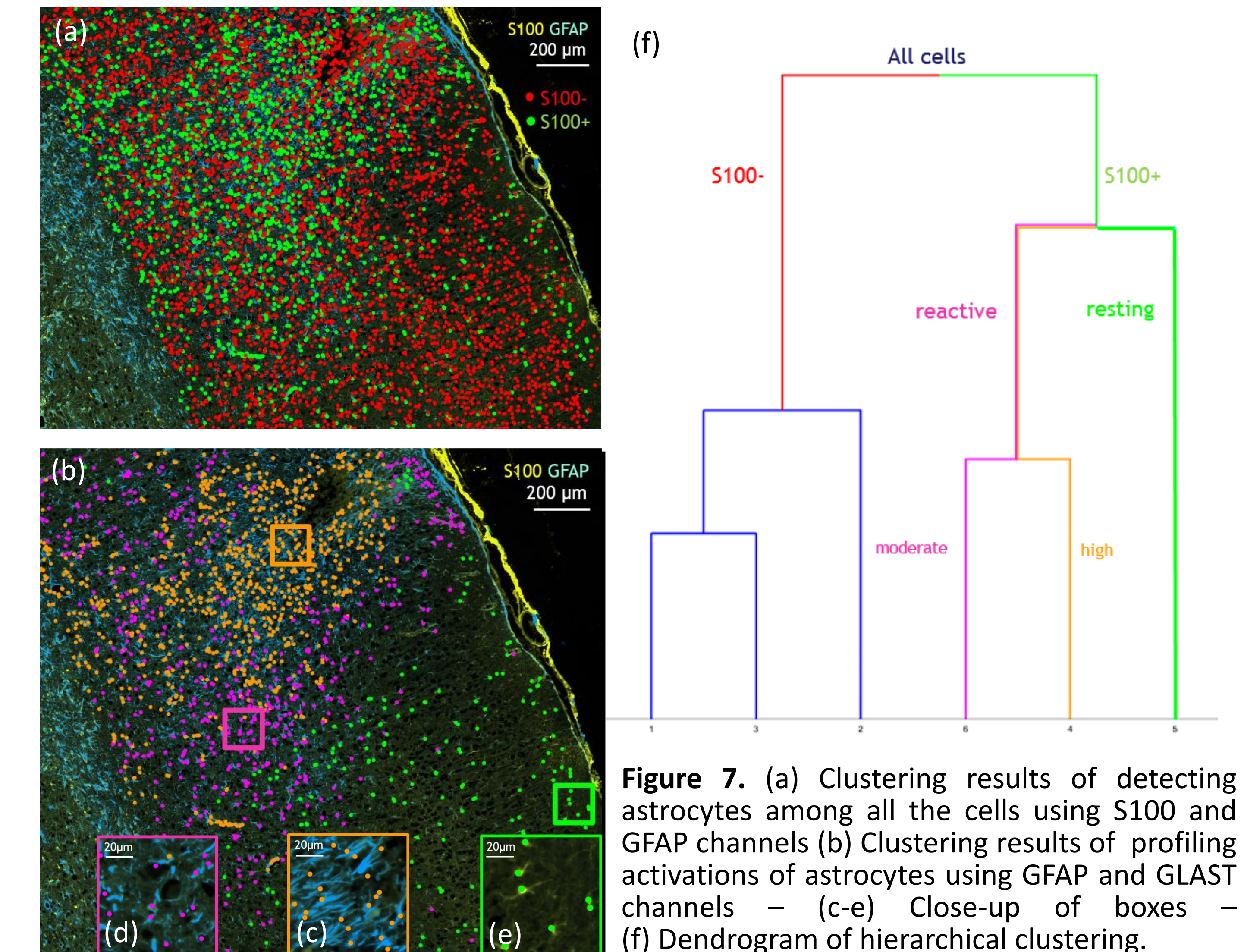


Figure 7. (a) Clustering results of detecting astrocytes among all the cells using S100 and GFAP channels (b) Clustering results of profiling activations of astrocytes using GFAP and GLAST channels – (c-e) Close-up of boxes – (f) Dendrogram of hierarchical clustering.

IMPACT: Enabled a deep profiling of astrocyte activation status in brain tissue in relation to each cell's location relative to the site of mild traumatic brain injury (mTBI).

CONCLUSIONS

- Developed a deep hierarchical clustering method for unsupervised profiling of cellular measurements that offers the following important advantages:
 - It is capable of seamlessly combining the molecular signature of each cell with its morphological signature in order to help discover biologically meaningful cell clusters.
 - It does not require extensive labeling and network training in order to compute the deep features, but delivers comparable performance. Instead, it uses a multiscale set of pre-defined wavelet based filters (Scattering Net) developed by Mallat. These features have the advantage of mathematical rigor unlike trained convolutional networks.
- Implemented a user friendly GUI (in MATLAB) that visualizes the mapping between the analysis results and the raw data:
 - The GUI takes advantage of the cluster distances depicted in the dendrogram to enable effective interpretation of the cell clusters by the user.

REFERENCES

- [1] Bogoslovsky, Tanya, et al. "Development of a systems-based in situ multiplex biomarker screening approach for the assessment of immunopathology and neural tissue plasticity in male rats after traumatic brain injury." *Journal of neuroscience research* 96.4 (2018): 487-500.
- [2] Ren, Shaoqing, et al. "Faster r-cnn: Towards real-time object detection with region proposal networks." *Advances in neural information processing systems*. 2015.
- [3] Bruna, Joan, and Stéphane Mallat. "Invariant scattering convolution networks." *IEEE transactions on pattern analysis and machine intelligence* 35.8 (2013): 1872-1886.