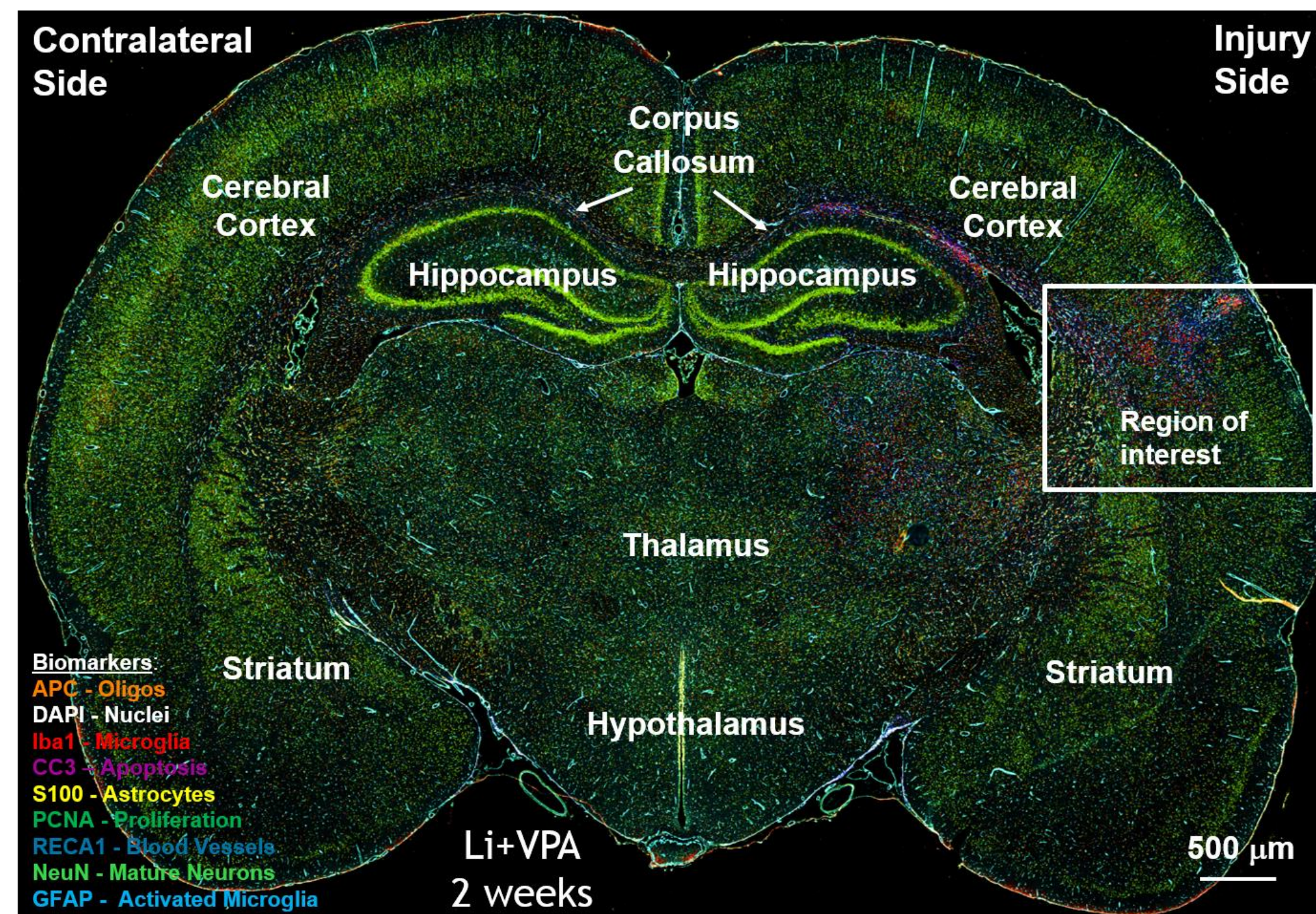


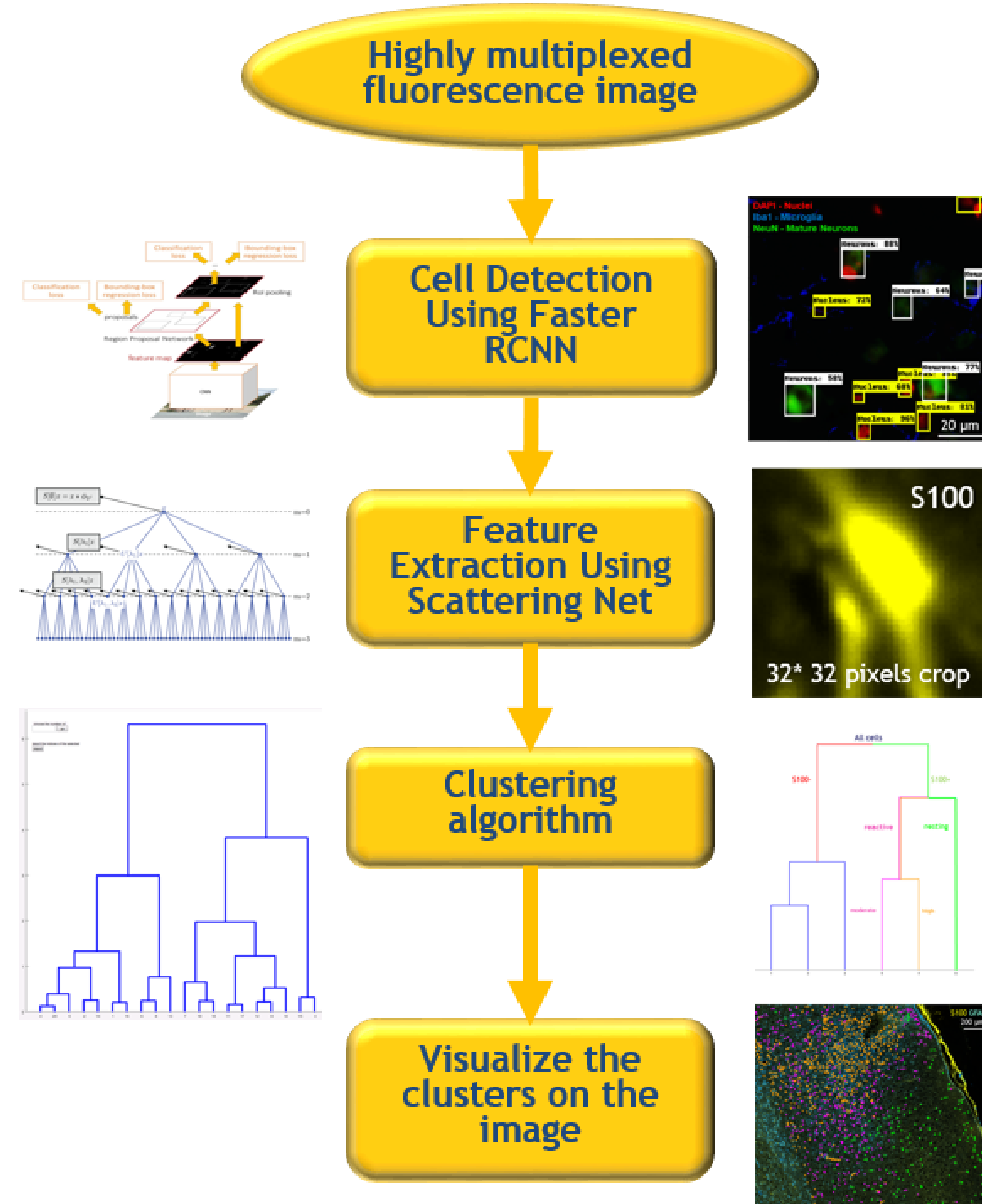
## OVERVIEW



**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 (+)

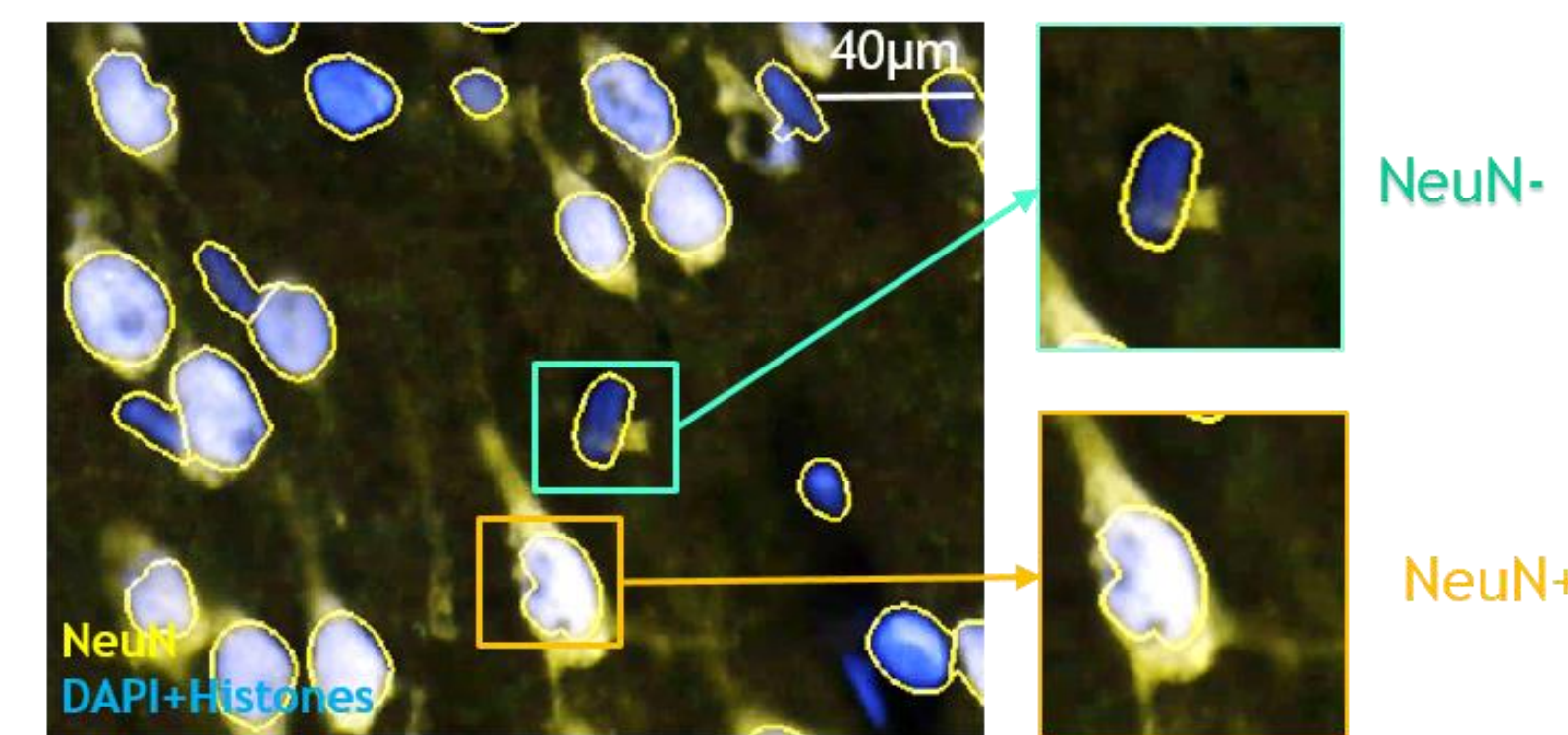
**Table 1.** Boolean logic table for cell type classification.



**Figure 2.** Image analysis pipeline.

## FEATURE EXTRACTION

### Conventional Cytometric Features:

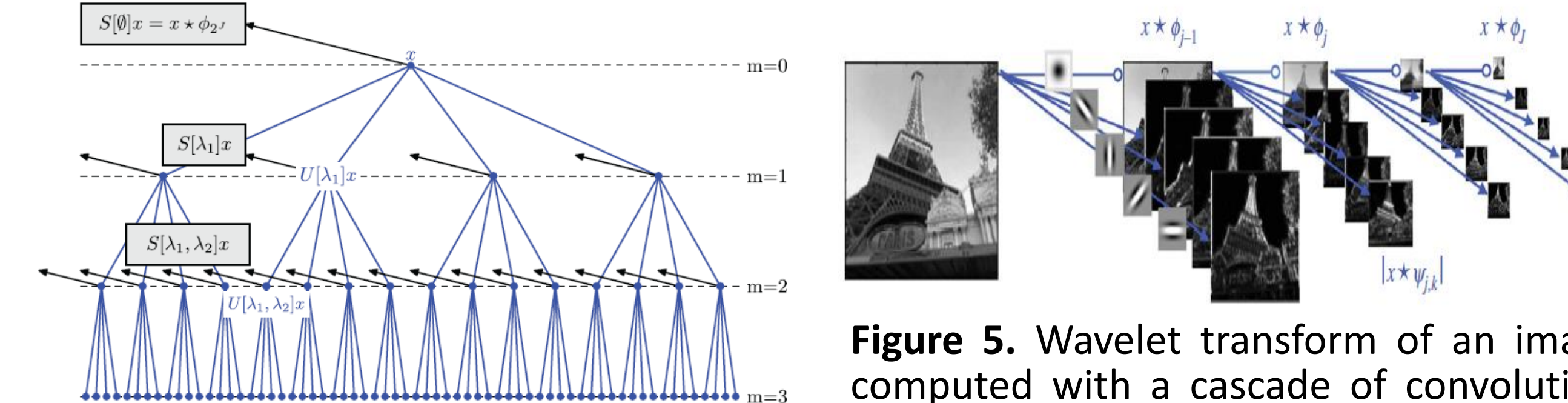


**Figure 3.** Nuclear segmentation of cells using DAPI +Histone channels.

### 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:

$$Wx(t) = \{x * \phi(t), x * \psi_\lambda(t)\}_\lambda$$

average      Convolution of signal with wavelets

2. Iteratively repeating the operator  $W$ .

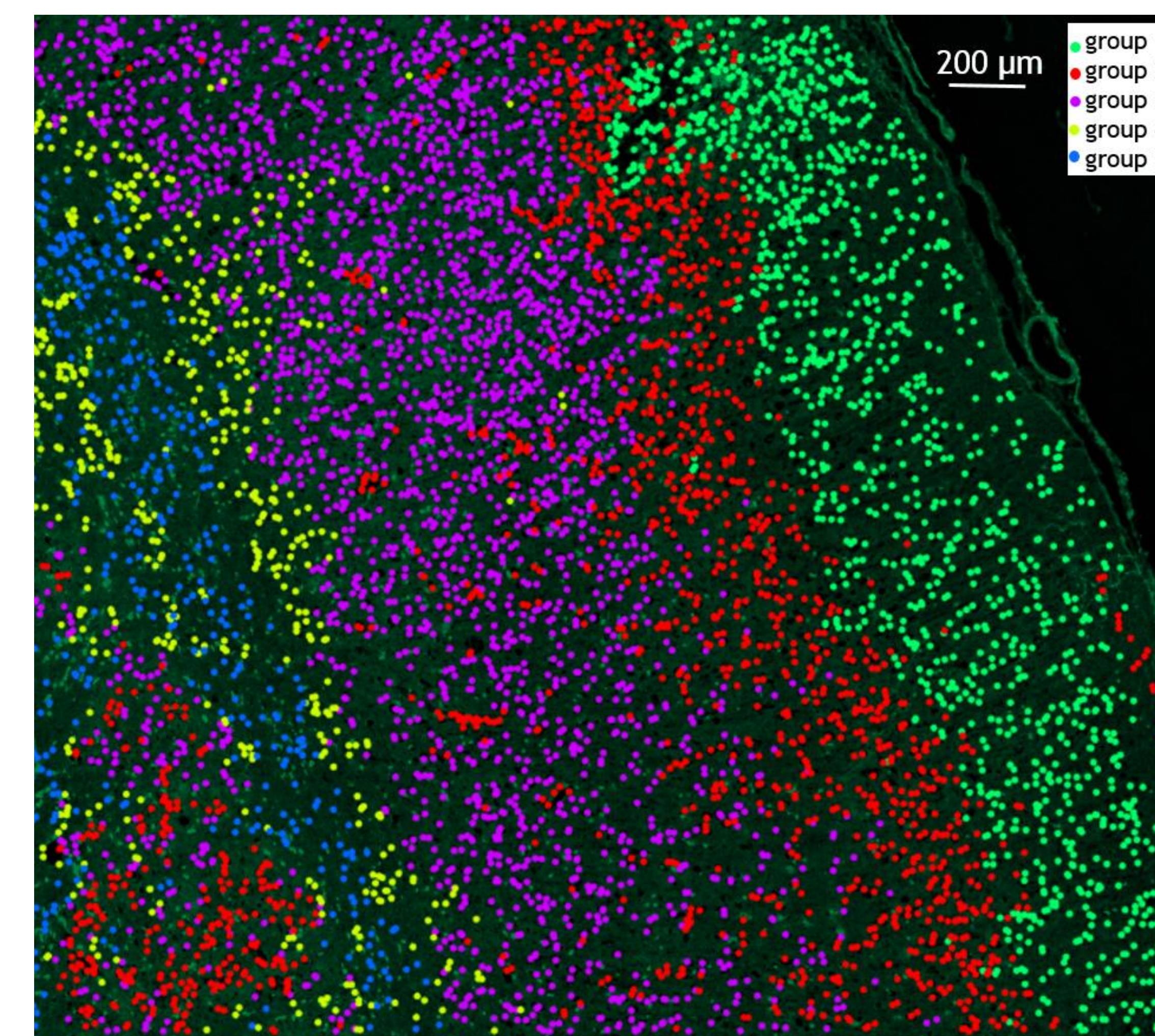
**build multi layers → deep network**

- ✓ 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

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

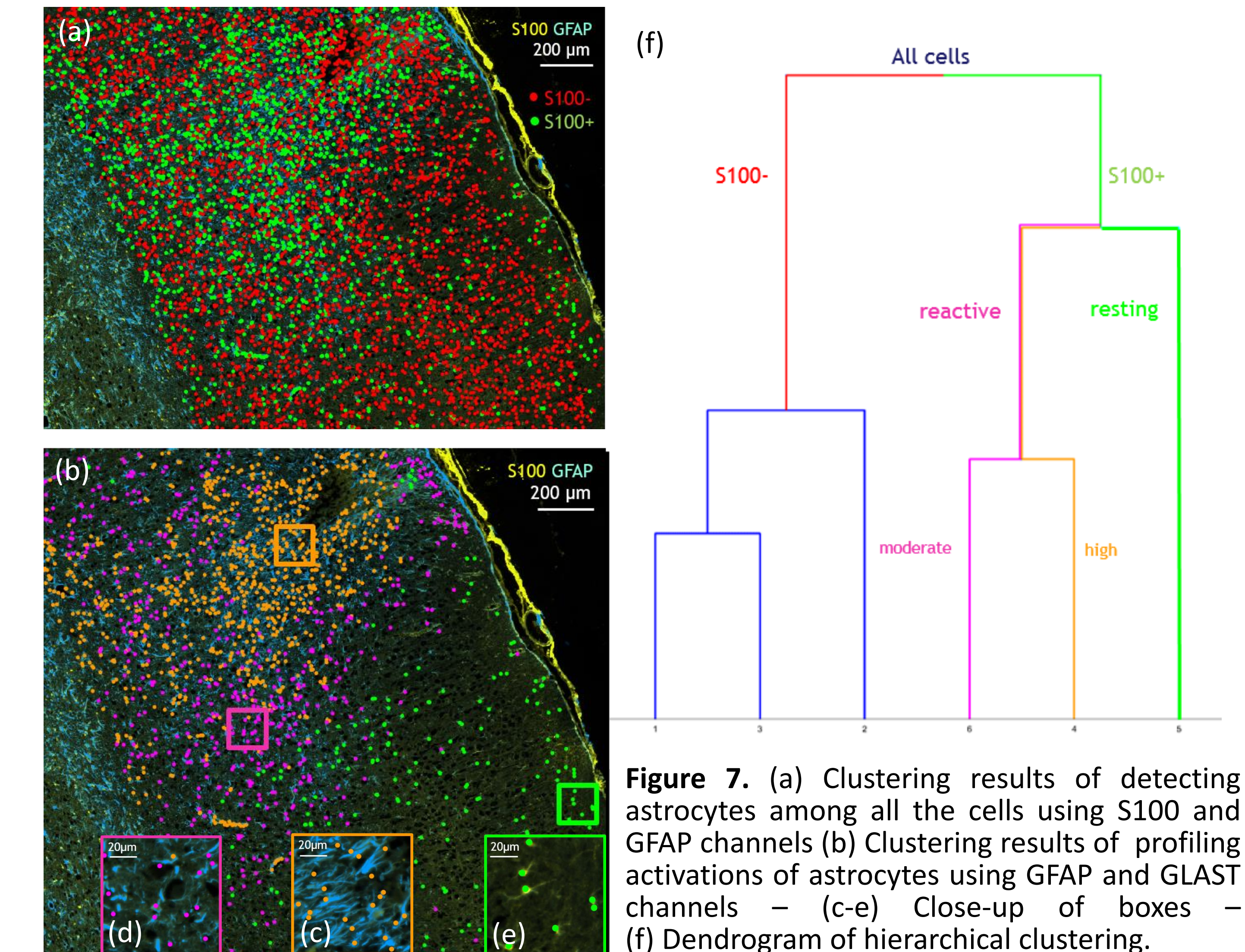
Overall Accuracy = 94.68%

		Predicted	
		-	+
Actual	S100 -	7,693	137
	S100 +	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.
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