Learning-based detection and segmentation of GFAP-labeled astrocytes in micrographs

Demetrio Labate (University of Houston)

Joint work with: Y. Huang, A. Kruyer, S. Syed, C. Kayasandik, M. Papadakis

SIAM TX-LA Section - November 6 2022

Outline

1. Astrocytes in the CNS
2. Automated quantitative analysis algorithms
3. Astrocyte detection
4. Astrocyte segmentation
5. Morphological analysis of astrocytes

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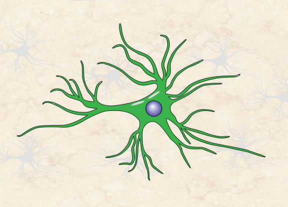

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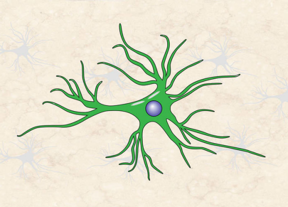

Astrocytes in the CNS

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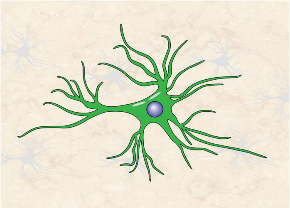
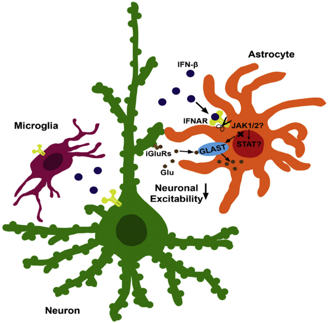
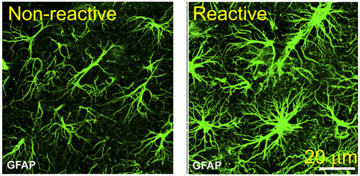
- Traditionally characterized as supportive cells (neuron homeostasis)
- Recent studies (past 5-10 years): active role in neuronal development and function (e.g., regulation of synaptic plasticity)





Astrocytes in the CNS

- Astrocytes reflect their diverse abilities and functions on their **complex morphology**



Images stained with Glial fibrillary acidic protein (GFAP) courtesy of Dr. Deneen, Baylor College of Medicine.

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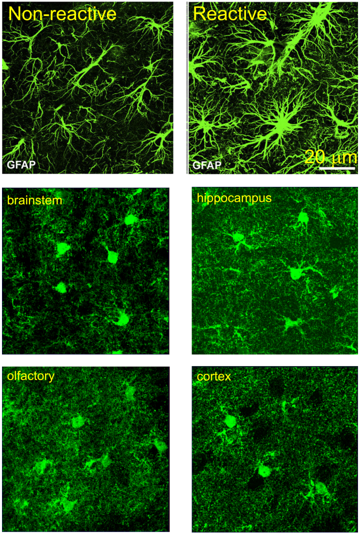

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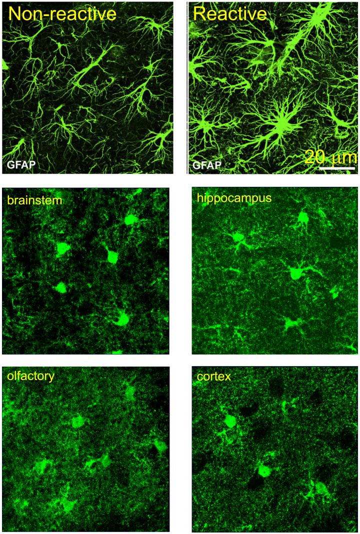

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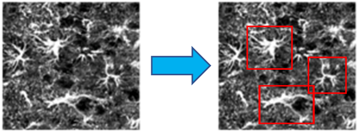

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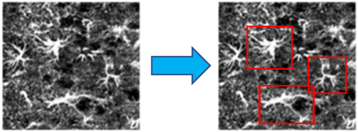

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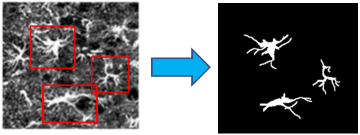

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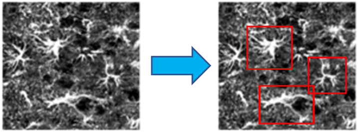

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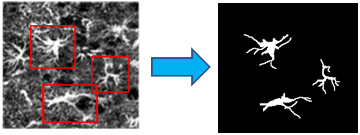
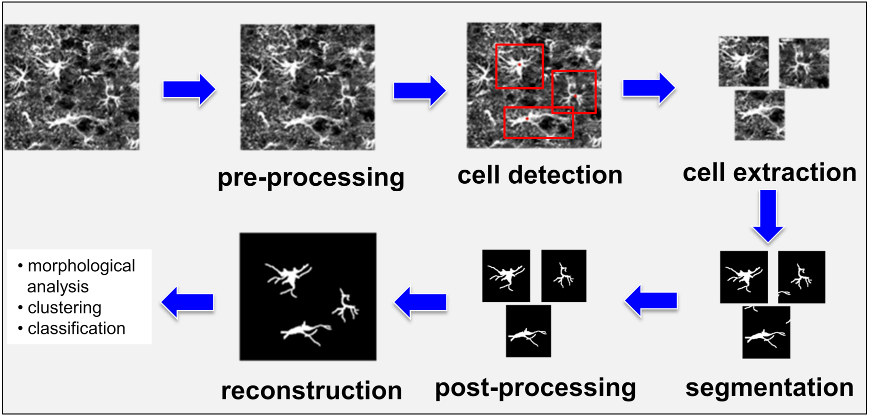


Following such processing tasks, one can carry out single-cell morphological analysis

Automated Analysis of Astrocytes

Image processing pipeline [Kayasandik at al, 2020]



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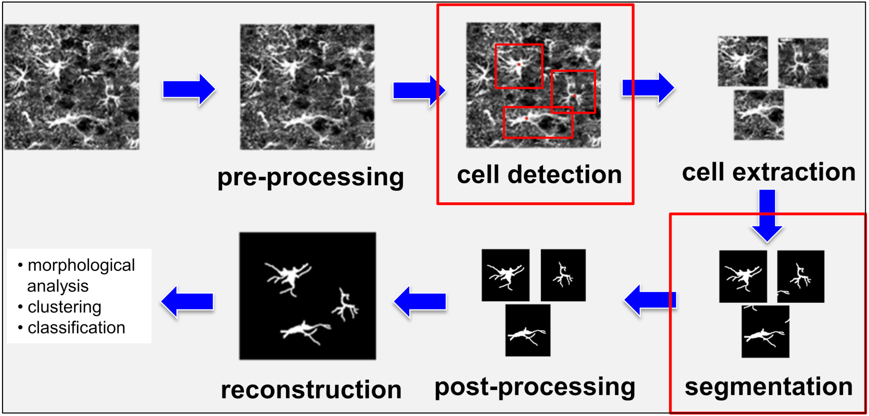
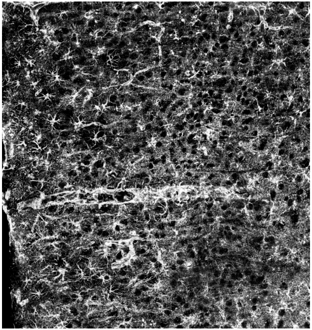
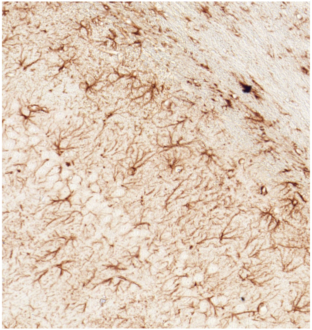


Analysis of Astrocytes

Automatic *detection* and *segmentation* of astrocytes is challenging due to complex shapes, morphological heterogeneity, entangled networks of cells.





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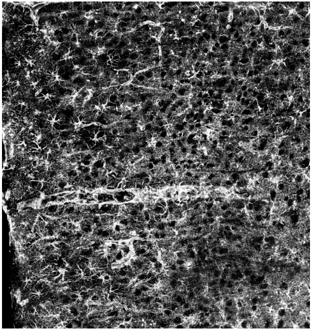

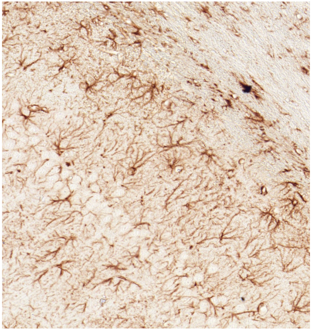

Algorithms developed for standard cell types perform poorly.

Automated analysis of astrocytes

Automated detection/segmentation of astrocytes in microscopy images:

- **Model-based methods**

PROS

- Performance guarantees
- Interpretability
- Data independent

CONS

- Computational intensive
- Limitations in handling complex rules

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- Lack of interpretation

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Astrocyte detection - model-based approach

Cell detection based on **Directional Ratio** [Kayasandik et al. 2019]

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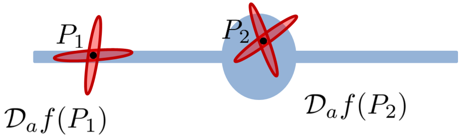
Consider a set of multiscale orientable filters $\{\phi_{j,\ell}\}$ associated with multiple scales and orientations.



- **Directional Ratio** of image f at the j-th scale and location p:

$$\mathcal{D}_j f(p) = \frac{\min_\ell\{|f * \phi_{j,\ell}(p)|\}}{\max_\ell\{|f * \phi_{j,\ell}(p)|\}}$$

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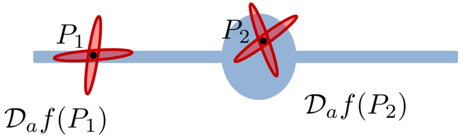

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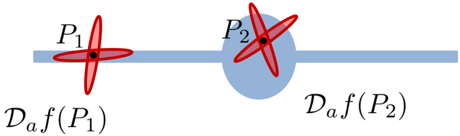

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- $\mathcal{D}_j f(p)$ discriminates points p based on the local isotropy of f near p.

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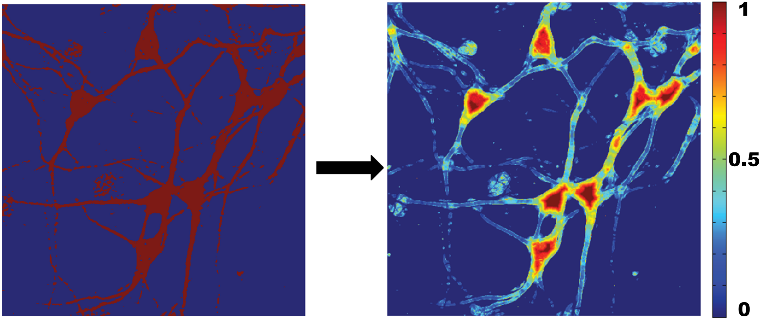
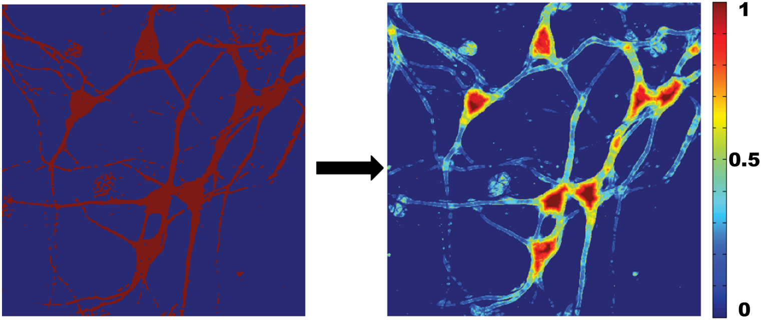
Result: There is a range of scales where the Directional Ratio is guaranteed to separates the two structures.



Computation of Directional Ratio

Astrocyte detection - Directional Ratio

- The Directional Ratio separates blob-like regions from elongated ones.



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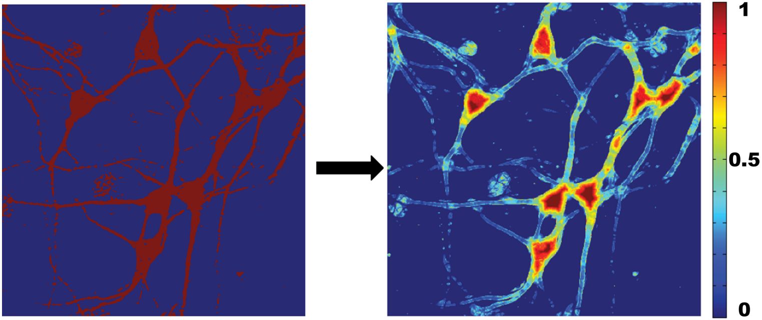

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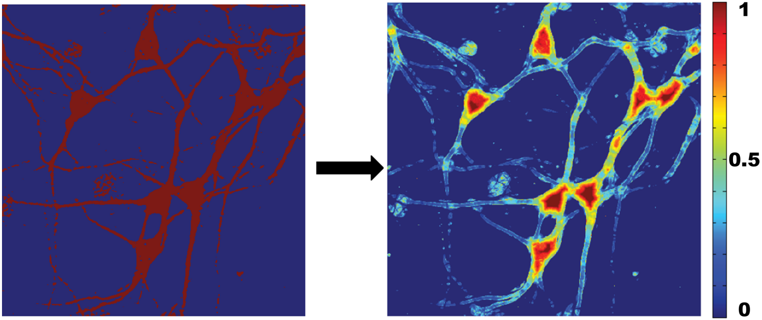

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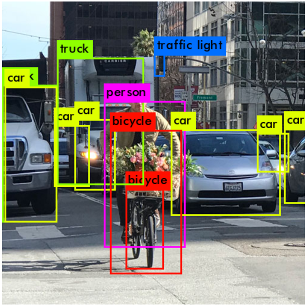
- It works on astrocytes provided there is a recognizable cell body
- useful to separate contiguous cells

Astrocyte detection - learning-based approach

Deep learning architectures have achieved state-of-the-art performance in many object detection tasks.

- **Faster R-CNN** (Ren et al, 2015; Microsoft Research)
- **DetectNet** (2016; NVIDIA), DetectNet v2 (2021)
- **YOLO** (Redmon et al, 2016), YOLOv2 (Redmon et al, 2017), YOLOv3 (Redmon, Farhadi, 2018), YOLOv4 (Bochkovskiy et al, 2020), YOLOv5 (Jocher, 2020; PyTorch)
- **RetinaNet** (Lin et al, 2017; Facebook AI Research)



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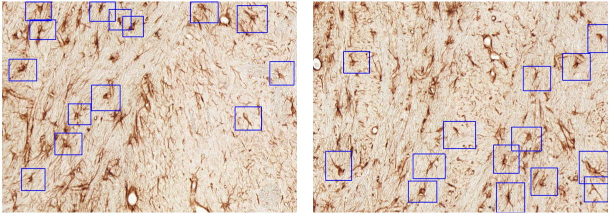
Authors created a dataset of 1120 annotated images including over 15,000 cells; released in the Broad Bioimage Benchmark Collection (BBBC).



Annotated GFAP-stained images in different rat brain regions.
(Bright field microscopy)

Astrocyte detection - YOLOv5

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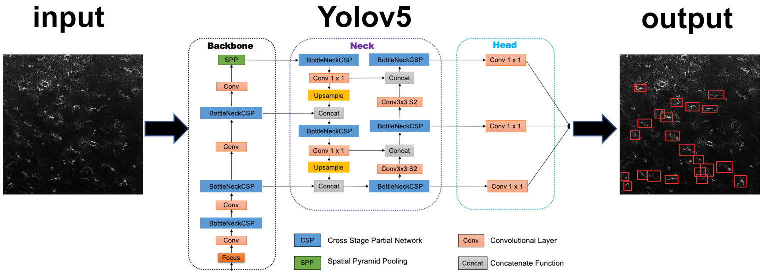

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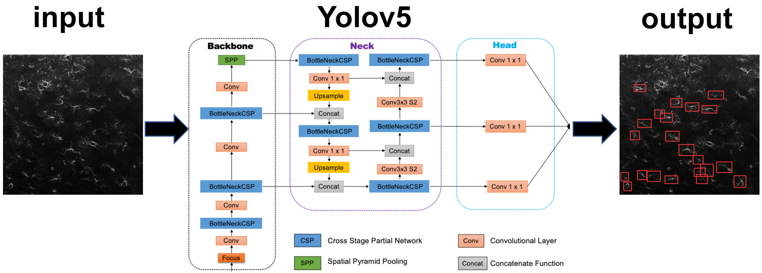

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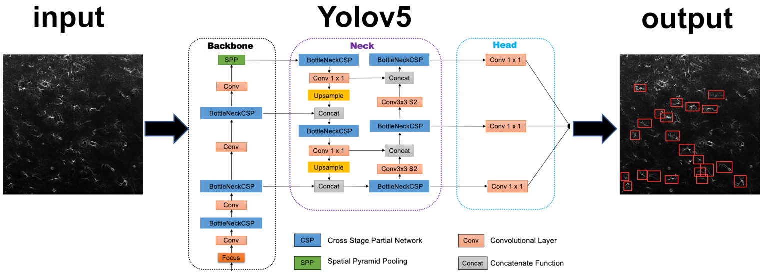

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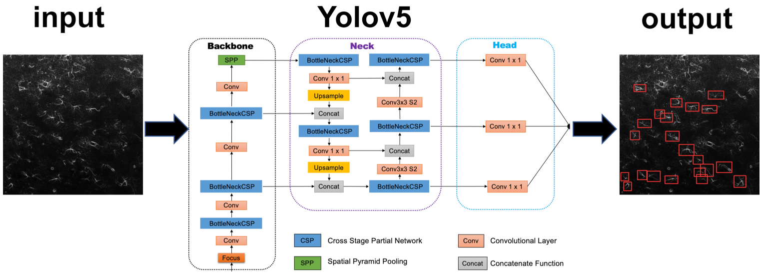

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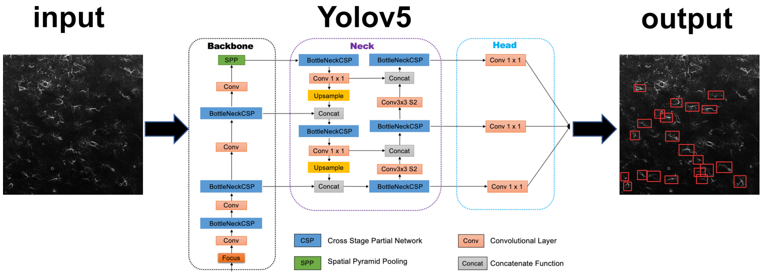

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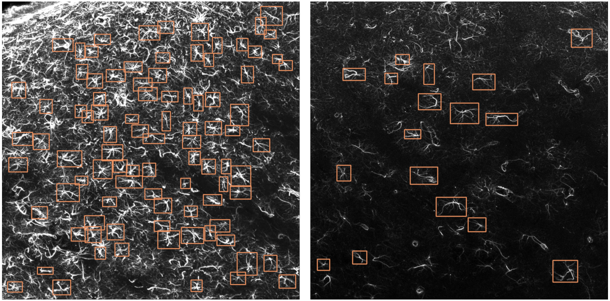
We selected a medium storage size model and optimized hyperparameters for our need.

Astrocyte detection - YOLOv5

We used a new set of GFAP-labeled images - cell preparation and image acquisition by Dr. Kruyer, Medical University of South Carolina.



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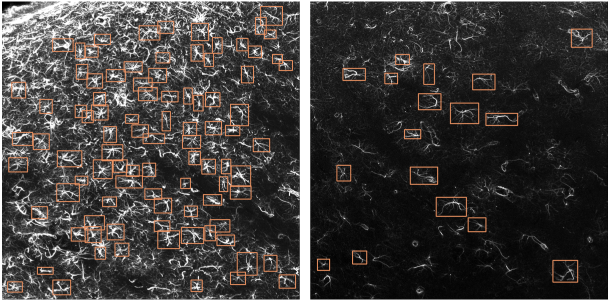

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Images include different conditions (Withdrawal, Relapse and Control).

Astrocyte detection - YOLOv5

Performance comparison for astrocyte detection on BBBC dataset

	YOLOv5+	**YOLOv5**	**DetectNet**	**D-Ratio**	**Ilastik**	**ImageJ**
P	0.90	0.83	0.86	0.59	0.30	0.54
R	0.82	0.76	0.78	0.87	0.67	0.31
F1	0.86	0.80	0.81	0.70	0.34	0.34

Comparison of our YOLOv5 approach against DetectNet [Suleymanova et al [2018], Directional ratio [Kayasandik et al, 2020], Ilastik [Berg et al, 2019] and ImageJ thresholding.

Performance metrics: Precision (P), Recall (R) and F1 score (F1)

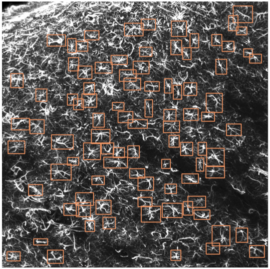
$$\mathrm{P} = \frac{\mathrm{TP}}{\mathrm{TP}+\mathrm{FP}}, \quad \mathrm{R} = \frac{\mathrm{TP}}{\mathrm{TP}+\mathrm{FN}}, \quad \mathrm{F1} = 2\,\frac{\mathrm{P}\cdot\mathrm{R}}{\mathrm{P}+\mathrm{R}}$$

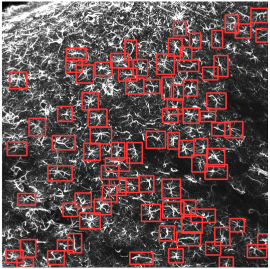
Astrocyte detection - YOLOv5

Performance comparison for astrocyte detection on new Kruyer dataset

	YOLOv5+	YOLOv5	DetectNet	D-Ratio	ImageJ
P	0.98	0.79	0.71	0.57	0.45
R	0.75	0.71	0.30	0.92	0.32
F1	0.85	0.74	0.42	0.71	0.37

Note: DetectNet was not re-trained on the new dataset.

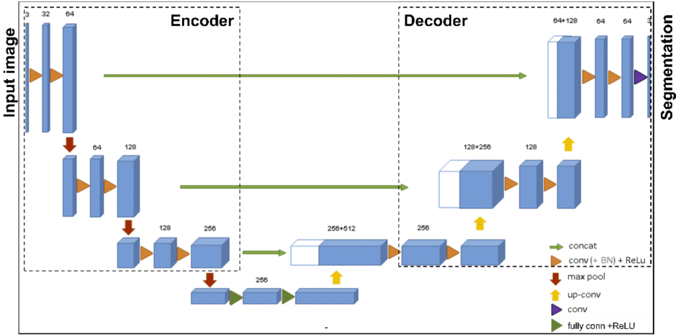




Ground truth (left) vs YOLOv5 detection (right)

Outline...

Deep learning algorithms provide state-of-the-art performance for cell segmentation.



U-net architecture [Ronneberger, Fischer, Brox, 2015]

A U-net combines an encoding feature-extracting section followed by a decoding section where pooling operations are replaced by upsampling.

Astrocyte segmentation - GESU-net

We introduced **Geometric-Enhanced Stacked U-net (GESU-net)** [Kayasandik et al, 2020]

- It stacks two U-nets to improve the recovery of finer processes.
  $\rightarrow$ [Ghosh, Arthita, et al. *Stacked U-Nets for ground material segmentation in remote sensing imagery.* Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition Workshops. 2018.]

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- To reduce the need of a large set of training data, we adopt the framework of Structured Receptive Field Neural Network.
  → cf [Jacobsen, Jorn-Henrik, et al. *Structured receptive fields in CNNs*. Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition. 2016.]

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- One novelty of our approach is the inclusion of specially designed filters and a sparsity constraint during training to reduce parametrization without losing expressive power.

Receptive Field Neural Networks

Receptive Field Neural Network (RFNN): convolution filters are expressed as **linear combinations from a fixed dictionary.** Only the coefficients of the linear combination are learned during training.

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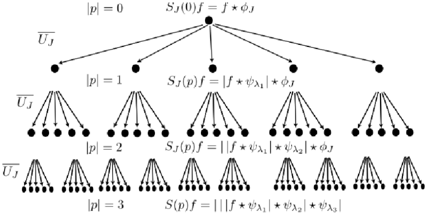
Inspired by **Scattering Transform [ST]** [Mallat, 2012]: a cascade of wavelet convolutional filters and nonlinearities.



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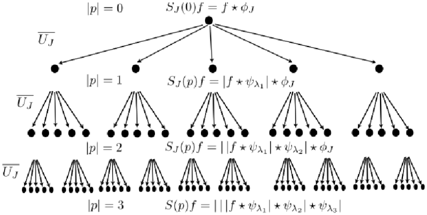

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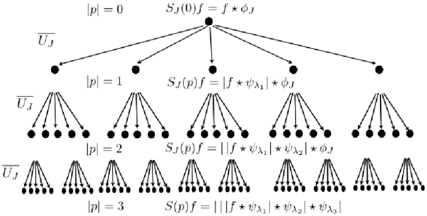
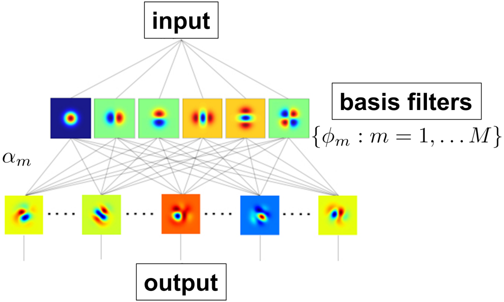


ST: Filters are **fixed**.

RFNN: Coefficients of the linear combination are **learned**.

Receptive Field Neural Networks

Each filter is of the form $f = \sum_{m=1}^{M} \alpha_m \, \phi_m$ where ϕ_m are fixed and the weights α_m are learned by the network.



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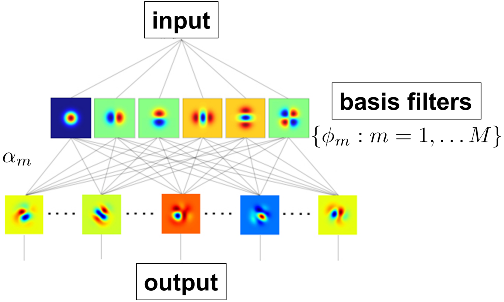

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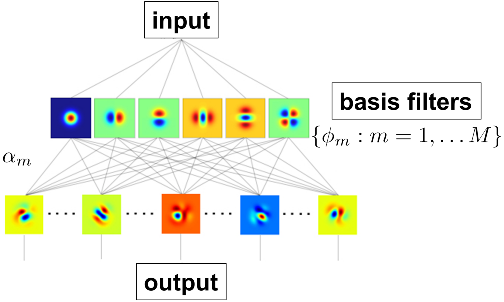

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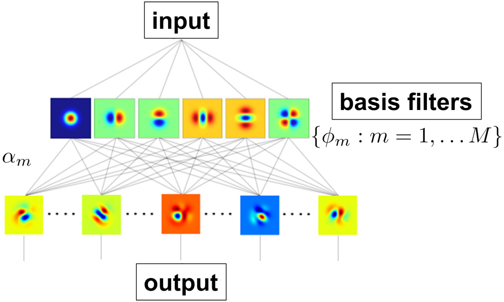

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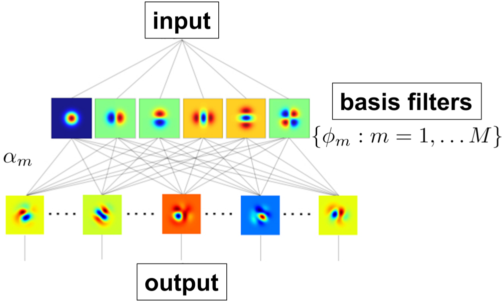
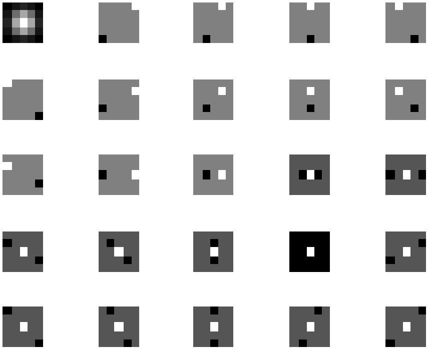


Advantages:

- Potentially less parameters than conventional CNN.
- Less prone to overfitting when limited training data is available.

Receptive Field Neural Networks

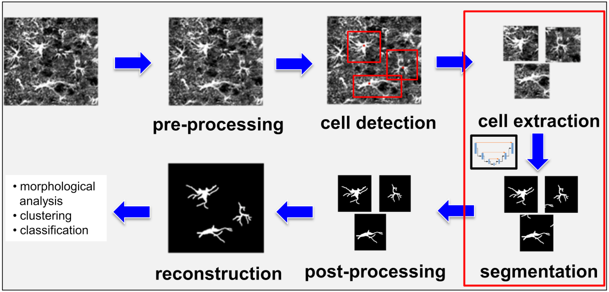
We design families of discrete compactly supported filters that form Parseval frames or almost Parseval frames and have additional geometrical properties, e.g., directional vanishing moments and sparsity [Atreas, Karantzas, Papadakis, Stavropoulos, 2018]



Parseval frame of 5×5 discrete filters.

Astrocyte segmentation - GESU-net

We apply our GESU-net to the sub-images obtained after the astrocyte detection processing step



Astrocyte segmentation - GESU-net

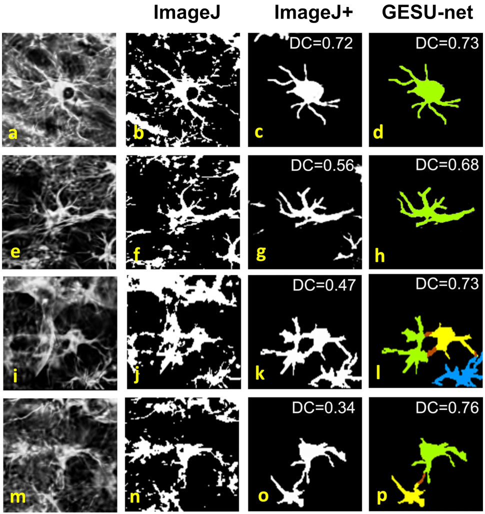
Performance comparison for astrocyte segmentation on a set of 65 astrocytes [Kayasandik et al 2020]

	GESU-net	**U-net+VGG16**	**U-net**	**ImageJ**	**ImageJ+**
P	0.86	0.58	0.55	0.46	0.61
R	0.69	0.87	0.77	0.62	0.69
F1	0.76	0.70	0.64	0.53	0.65

Comparison of our GESU-net against a conventional U-net, a U-net with a pre-trained VGG module, an thresholding method in ImageJ and an improved thresholding method combined with our detection method.

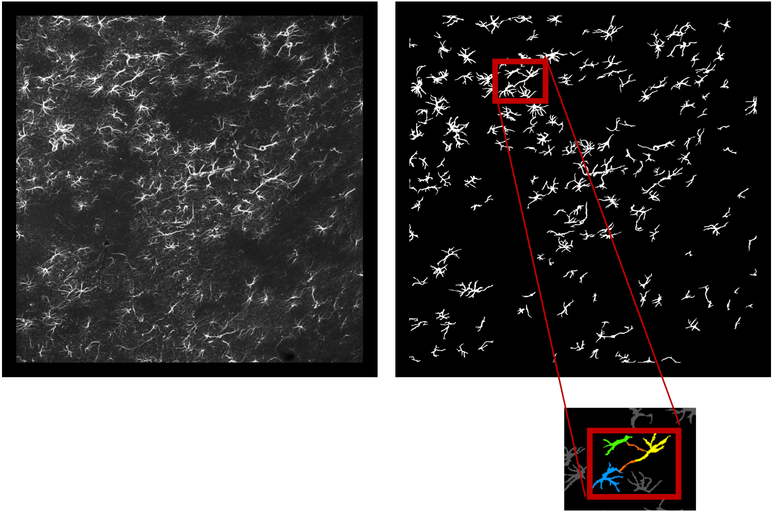
Astrocyte segmentation - GESU-net

Visual comparison



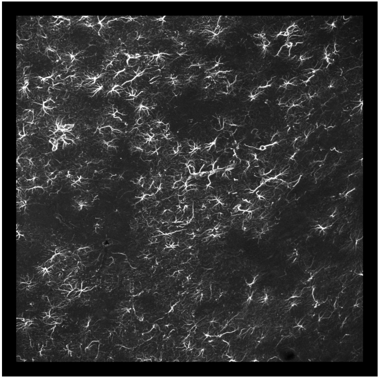
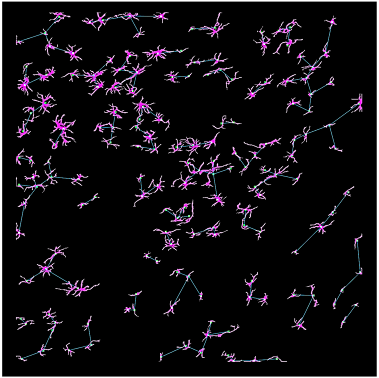
Astrocyte segmentation - GESU-net

Full image segmentation



Astrocyte segmentation - GESU-net

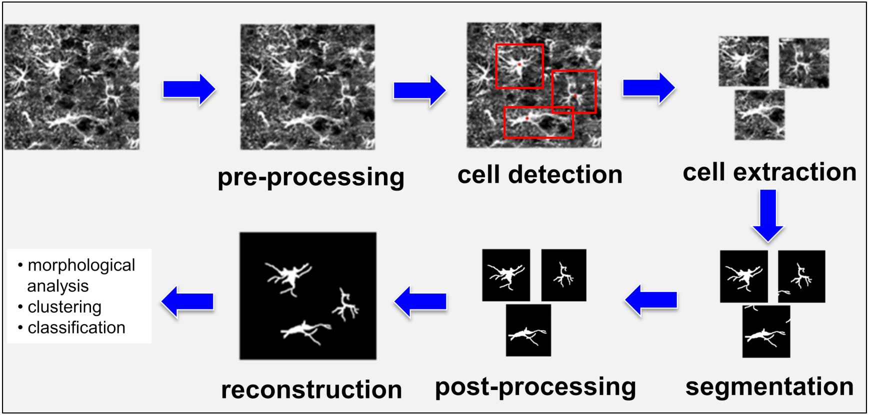
Full image segmentation

Outline...

Image-based Analysis of Astrocytes

Automated image processing pipeline



- Modular design

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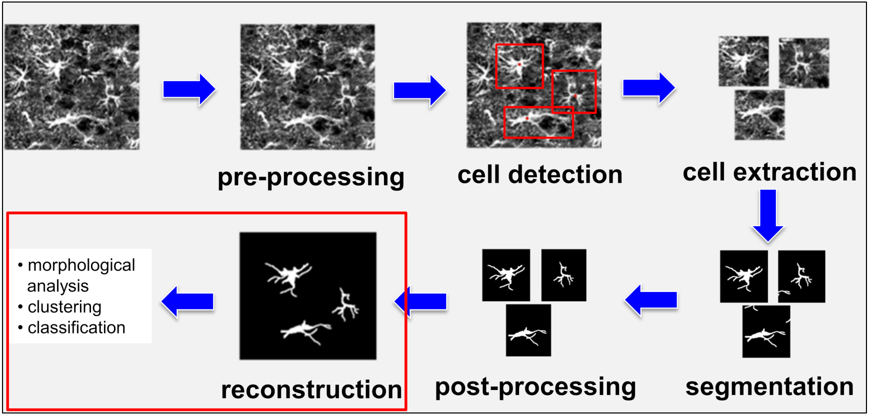
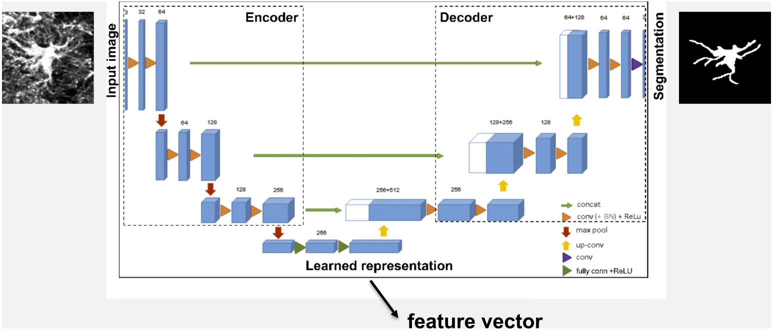


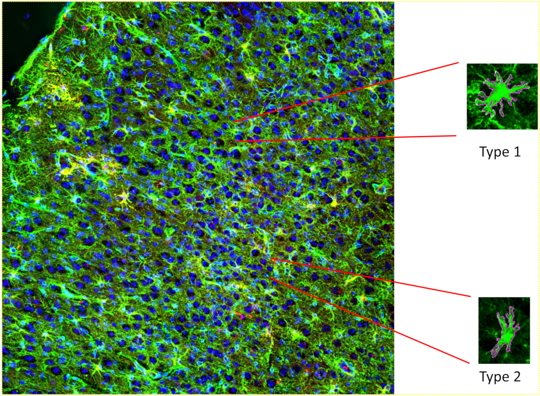
- Modular design

Representation learning: feature selection of astrocyte morphology



As the network learns to segment a cell, so it learns a **representation of the cell morphology** that is encoded in the saddle section of the network.

Image-based Analysis of Astrocytes



Green (GFAP): astrocyte cells, red: post-synapse marker, blue (DAPI): nuclei.

- Post-synapses expression indicates presence of segregated populations.

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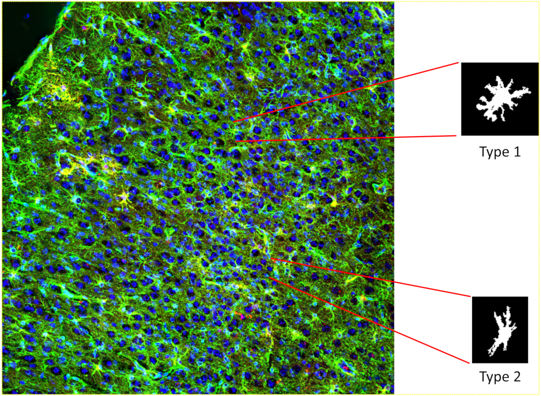

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• Supervised classification using of image-based features astrocyte morphology shows high correlation with functional phenotype.

References + codes at: `www.math.uh.edu\~dlabate`

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