Human Brain Project

EBRAINS

SENSEI: uncovering neuron structure using hard and soft approaches

Nicola Vanello, Research Center E. Piaggio, Univ. of Pisa

HBP Partnering Projects Meeting: Status quo & outlook

5-7 September 2022 | Nijmegen, The Netherlands

Co-funded by
the European Union

SENSEI Goal

Obtaining neuronal morphology at different spatial levels from optical imaging techniques

- e.g. dendritic tree & subcellular structures/spines

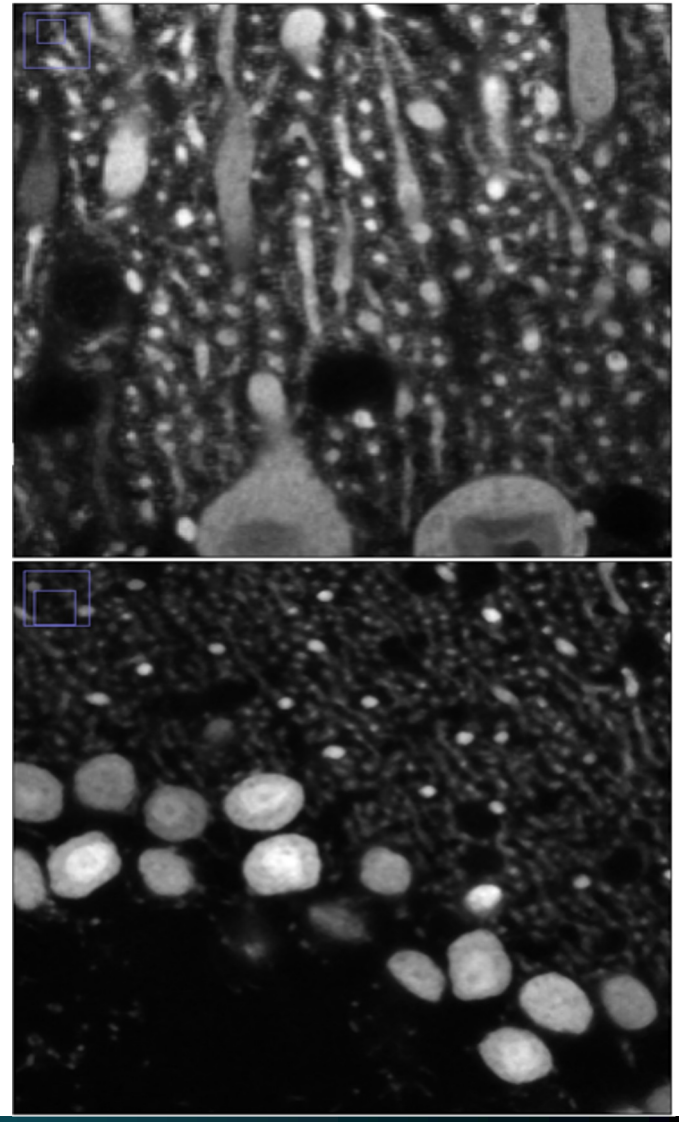
Tools:

Tissue Processing and Imaging

Segmentation algorithms

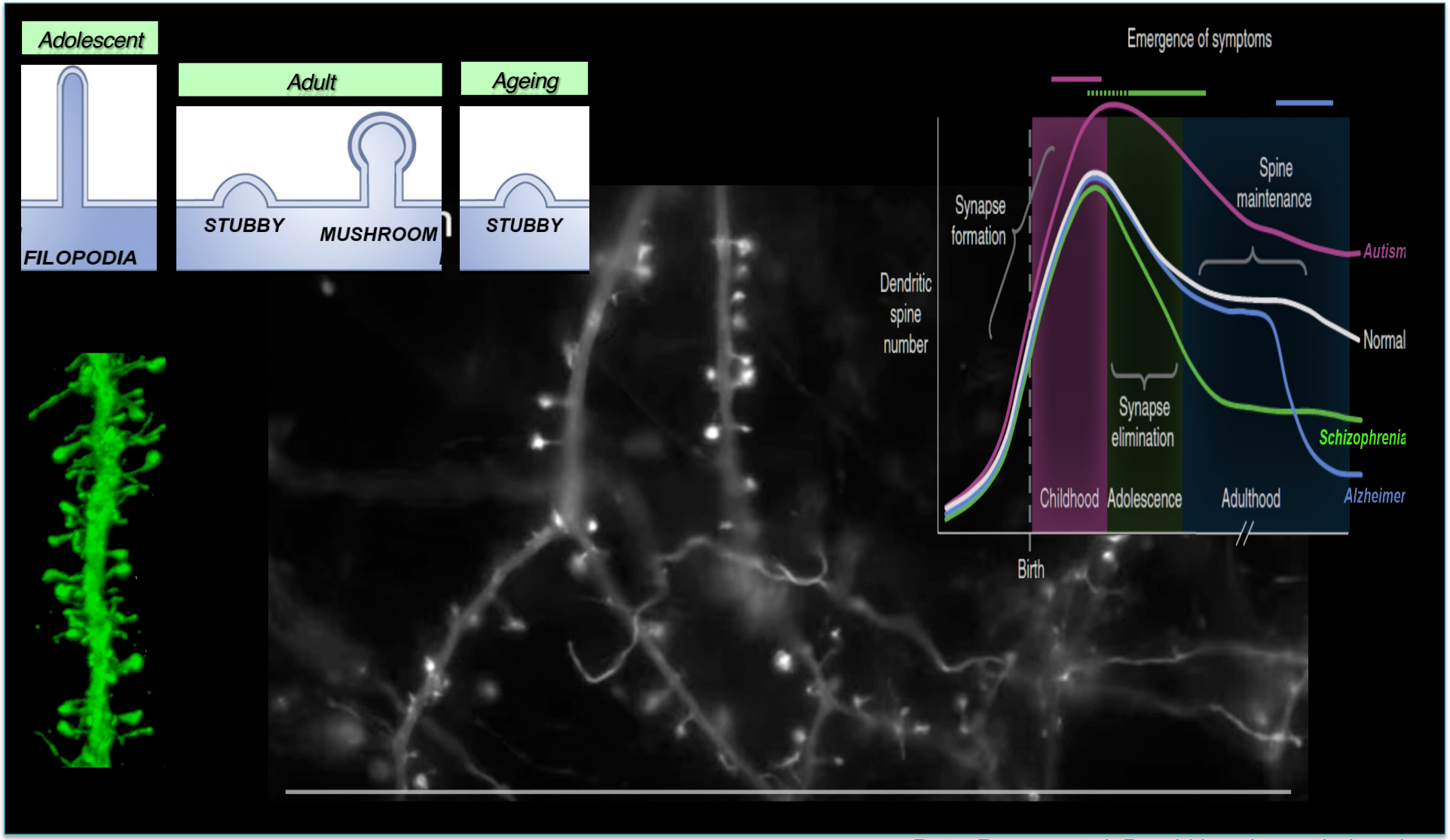
- model based approaches
- k-means exploiting topological information

TriScan: very fast 3D imaging fluorescence microscope



Co-funded by the European Union

Dendritic spine morphology is associated to learning & ageing



Maria Fischer, et al, Rapid Actin-Based Plasticity in Dendritic Spines, Neuron, Volume 20, Issue 5, 1998

Peter Penzes et al. Dendritic spine pathology in neuropsychiatric disorders Nat Neurosci. 2011 Mar; 14(3): 285–293.

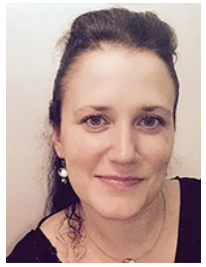
SENSEI Team

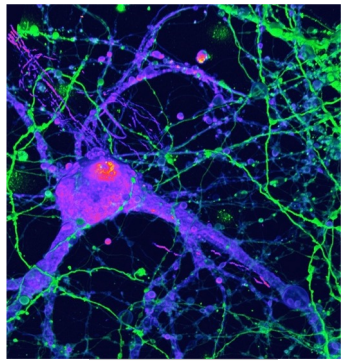
SEgmentation of Neurons using Standard and supEr-resolution mIcroscopy

Inserm

ipnp

Labelling & Microscopy

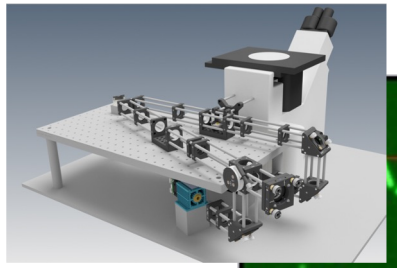


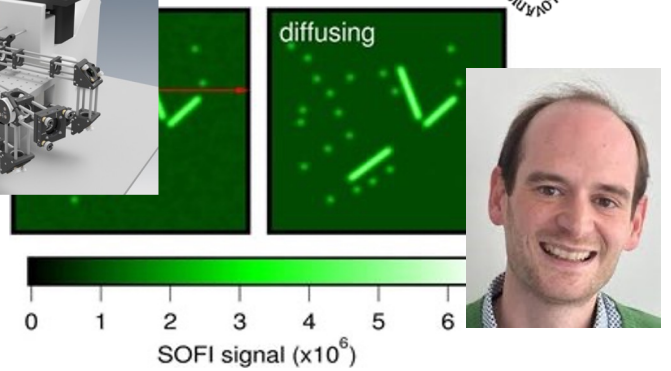


Lydia Danglot

ANR

Triscan Device 3D SOFI





Peter Dedecker

fwo

Segmentation algorithms

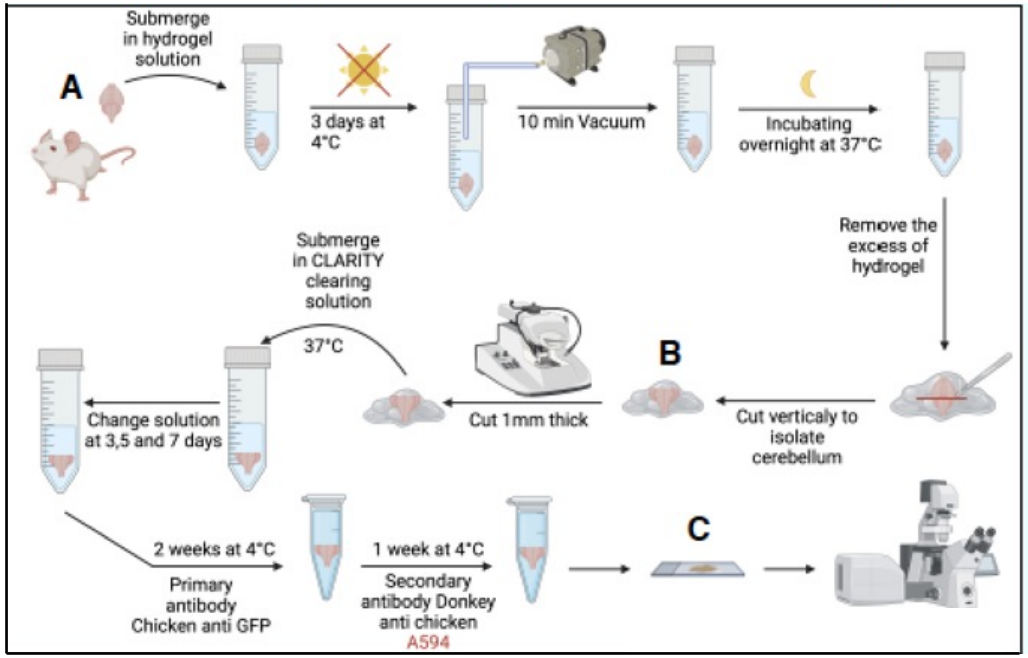
Centro E. Piaggio

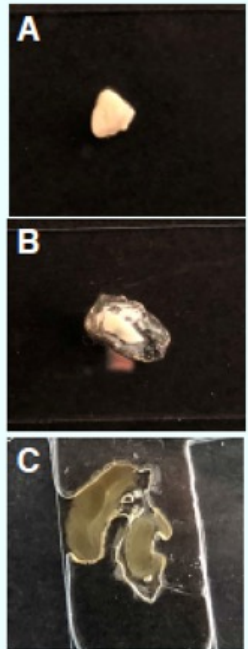
MUR

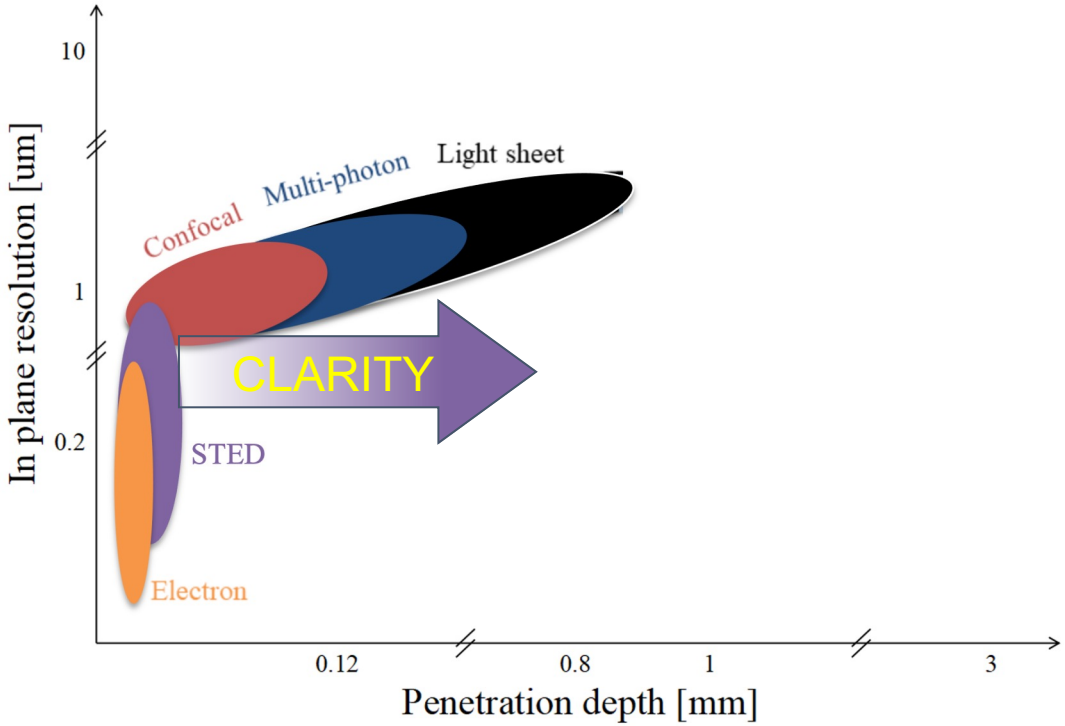
FLAG-ERA

Co-funded by the European Union

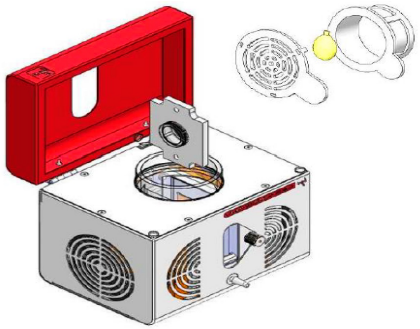
Labeling Strategy to decipher neural shape within tissue slices

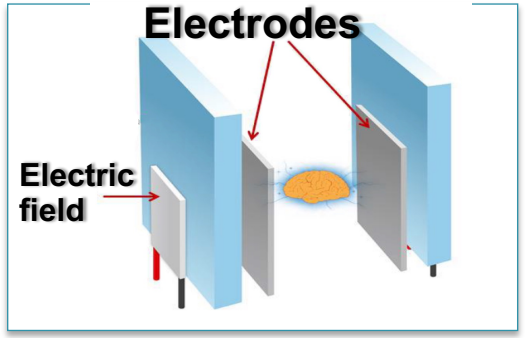


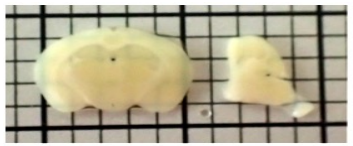


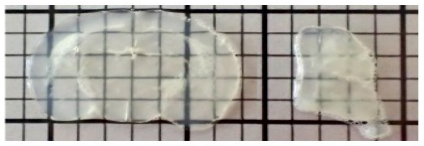


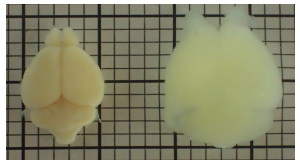
Tissue Clarification

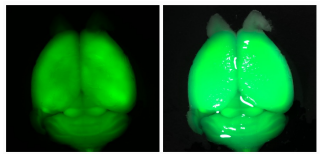




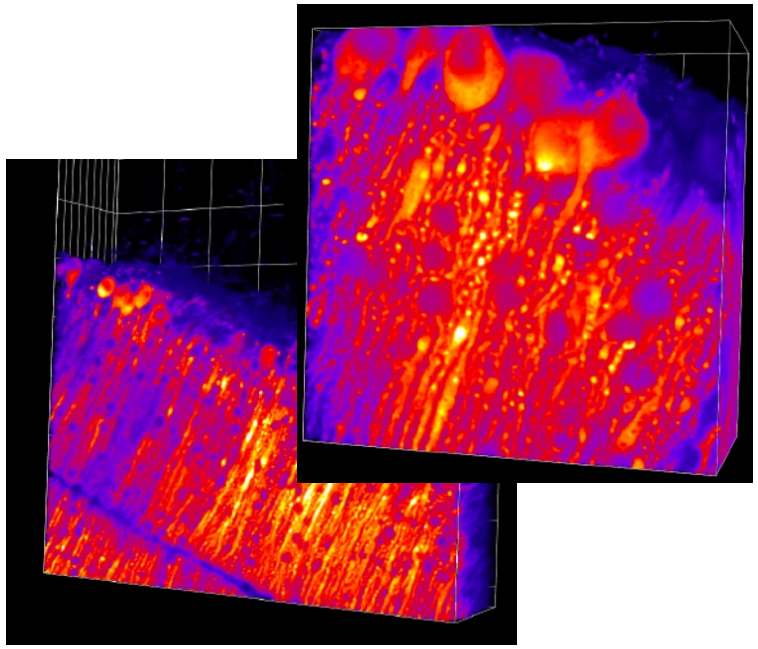




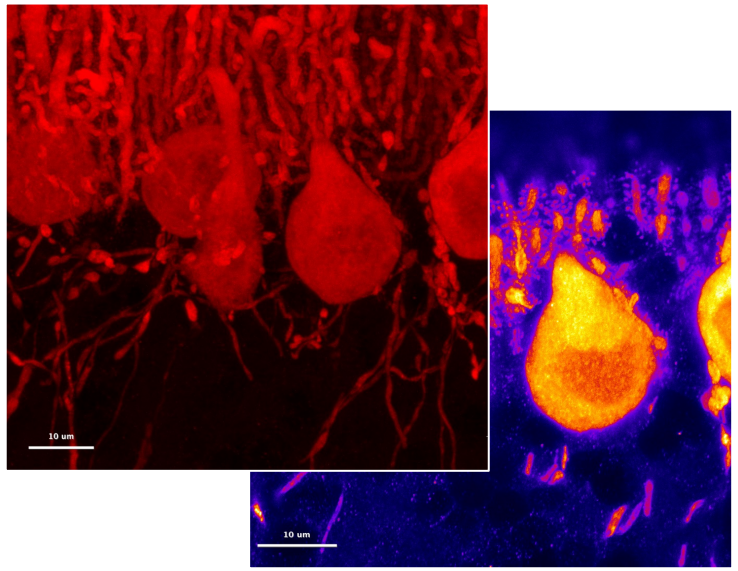




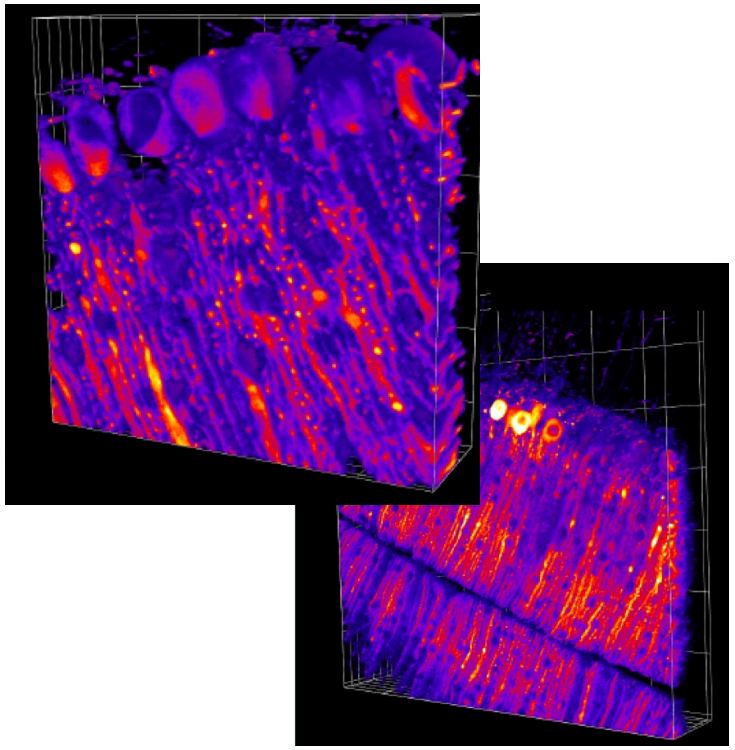
Acquisitions with different microscopy modalities



Spinning disk
20x 63x



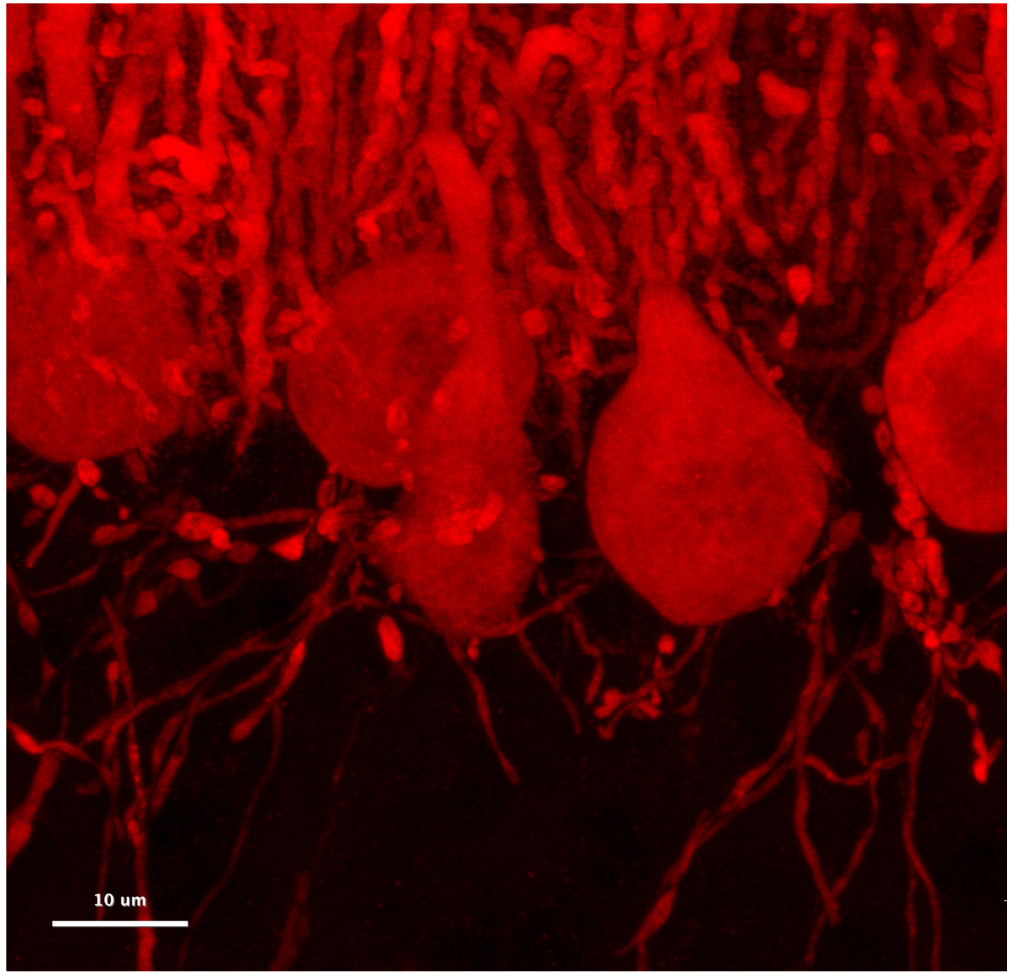
Confocal Leica/3D sted
93x



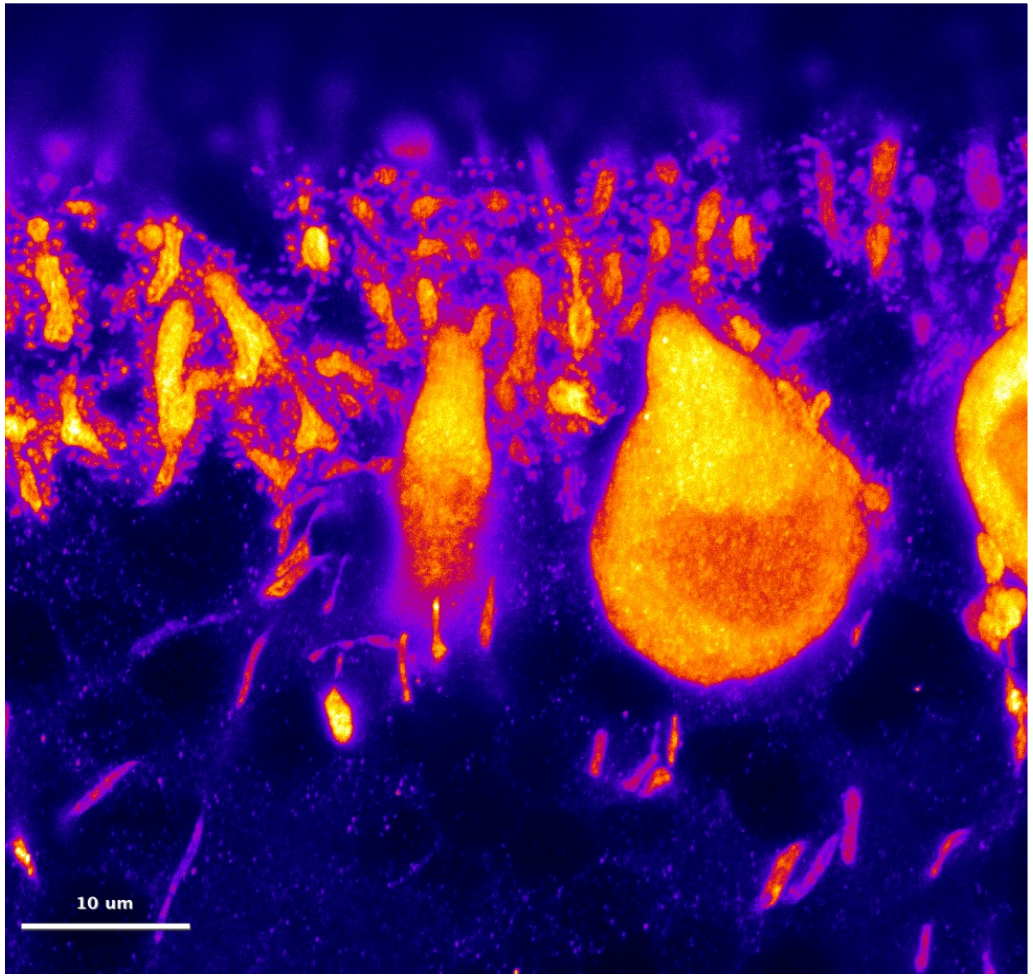
Confocal Leica
63x/25x

Images acquired with different modalities are acquired and will be analyzed with proposed segmentation approaches

Acquisitions with different microscopy modalities



Confocal 93x- 3D projection



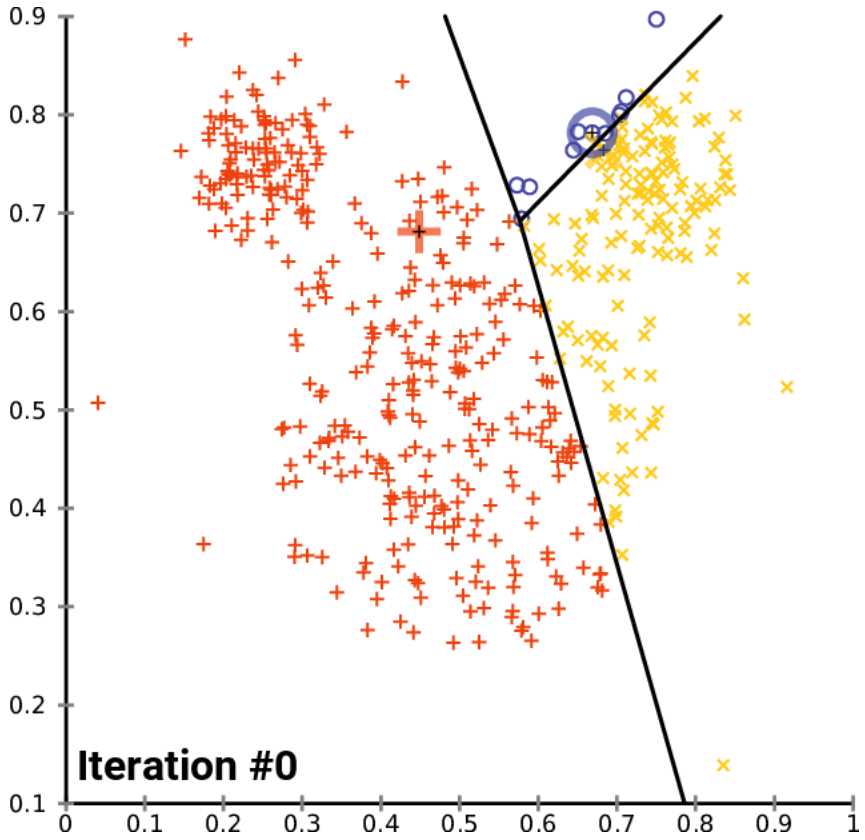
3D STED 93x – 3D projection
spine morphology even in thick slices (500 μm)

SENPAI: a topological informed data driven approach

A k-means algorithm exploiting spatial derivatives

K-means clustering, needs

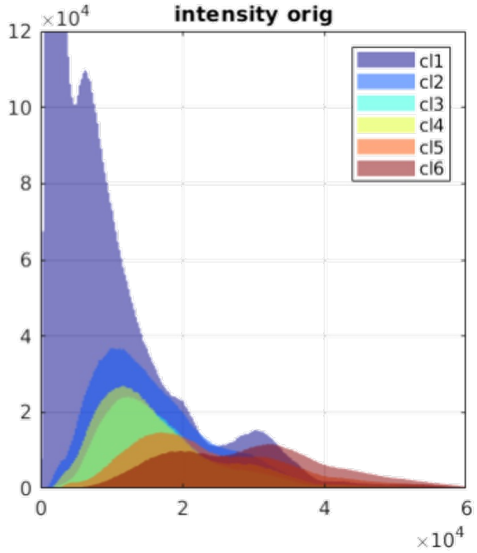
the number of possible classes
a definition of distance
the criterion (i.e. minimization of within cluster variance)



https://commons.wikimedia.org/wiki/File:K-means_convergence.gif

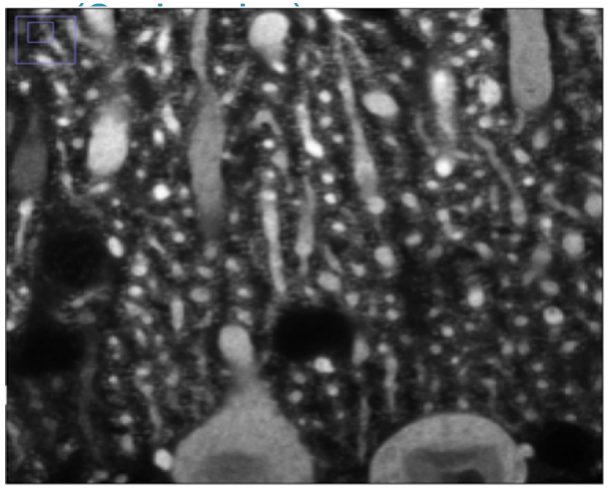
SENPAI: a topological informed data driven approach to neuronal reconstruction

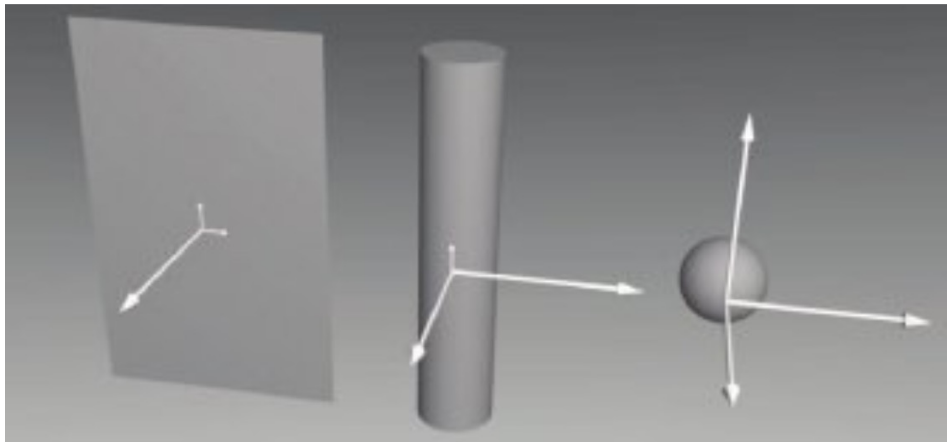
A k-means algorithm exploiting spatial derivatives



Intensity distribution of pixel classes; they overlap

If we use spatial derivatives

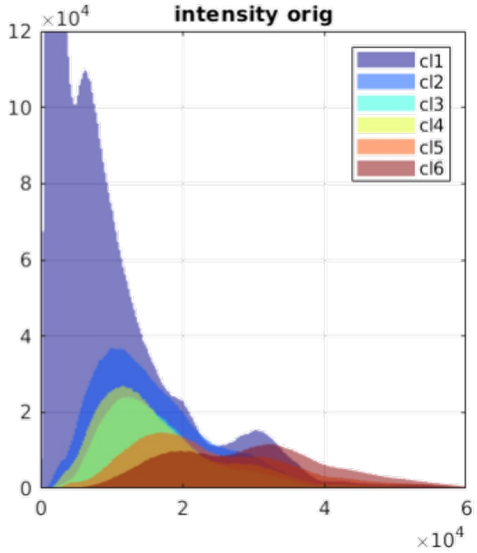




The analysis with Hessian allows to describe different spatial distributions

SENPAI: a topological informed data driven approach to neuronal reconstruction

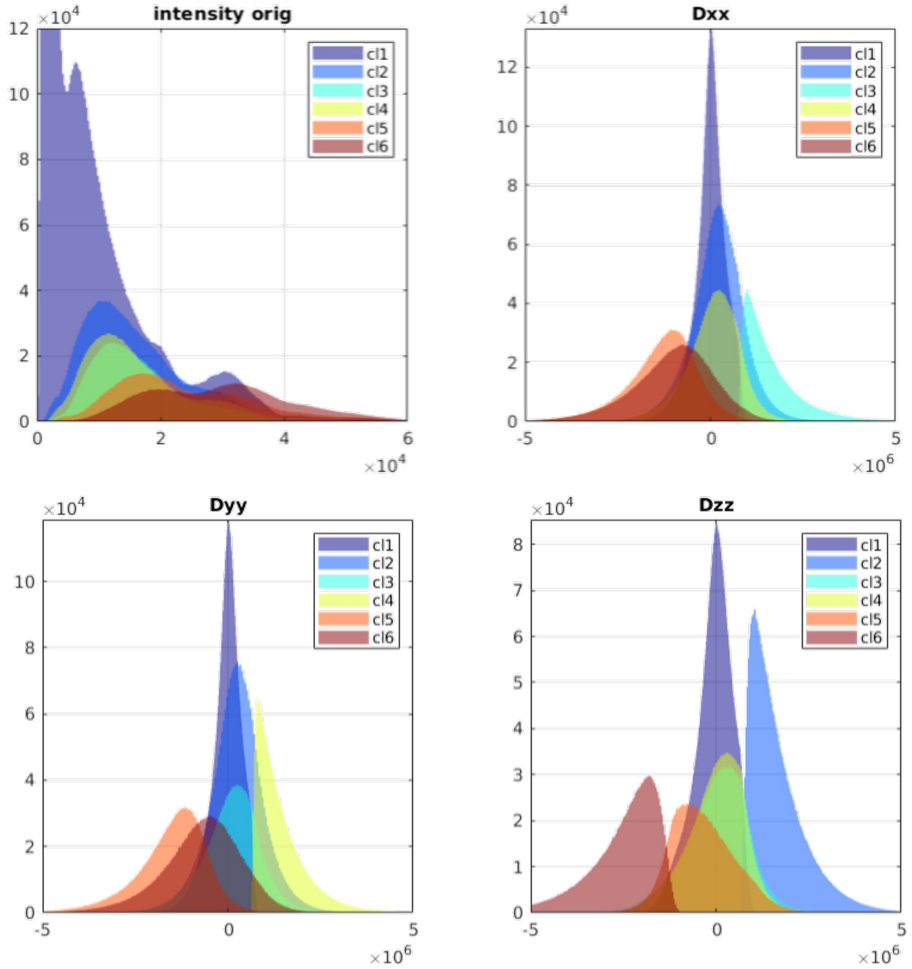
A k-means algorithm exploiting spatial derivatives



Intensity distribution of pixel classes; they overlap

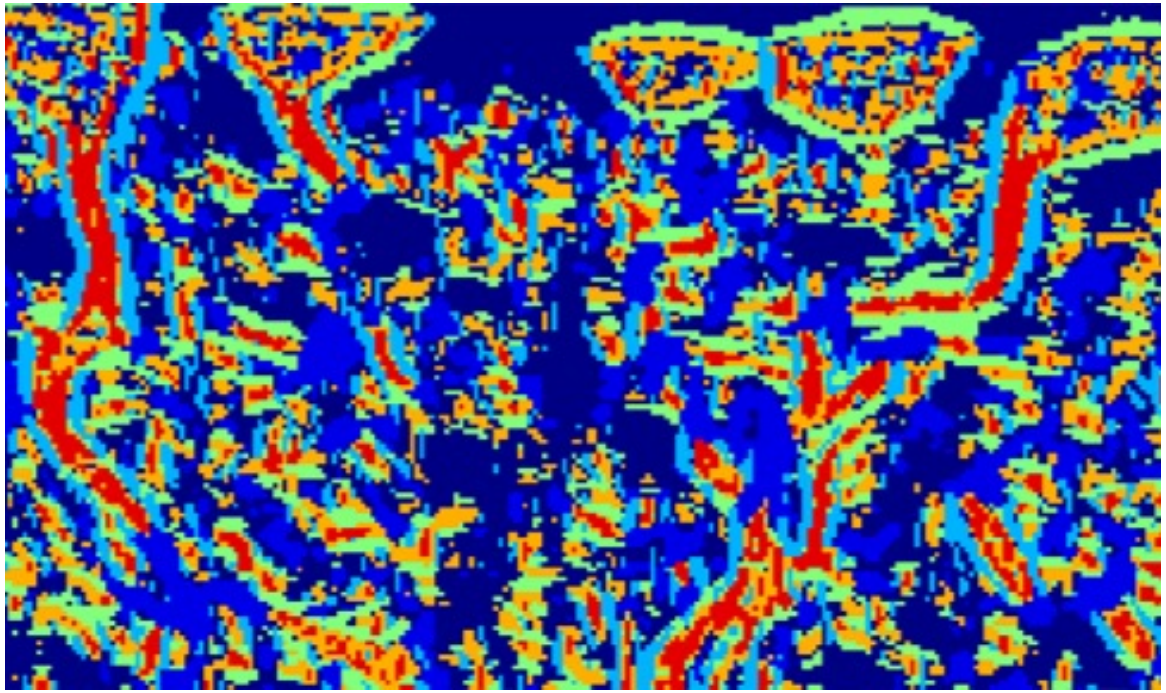
If we use spatial derivatives (2nd order)…

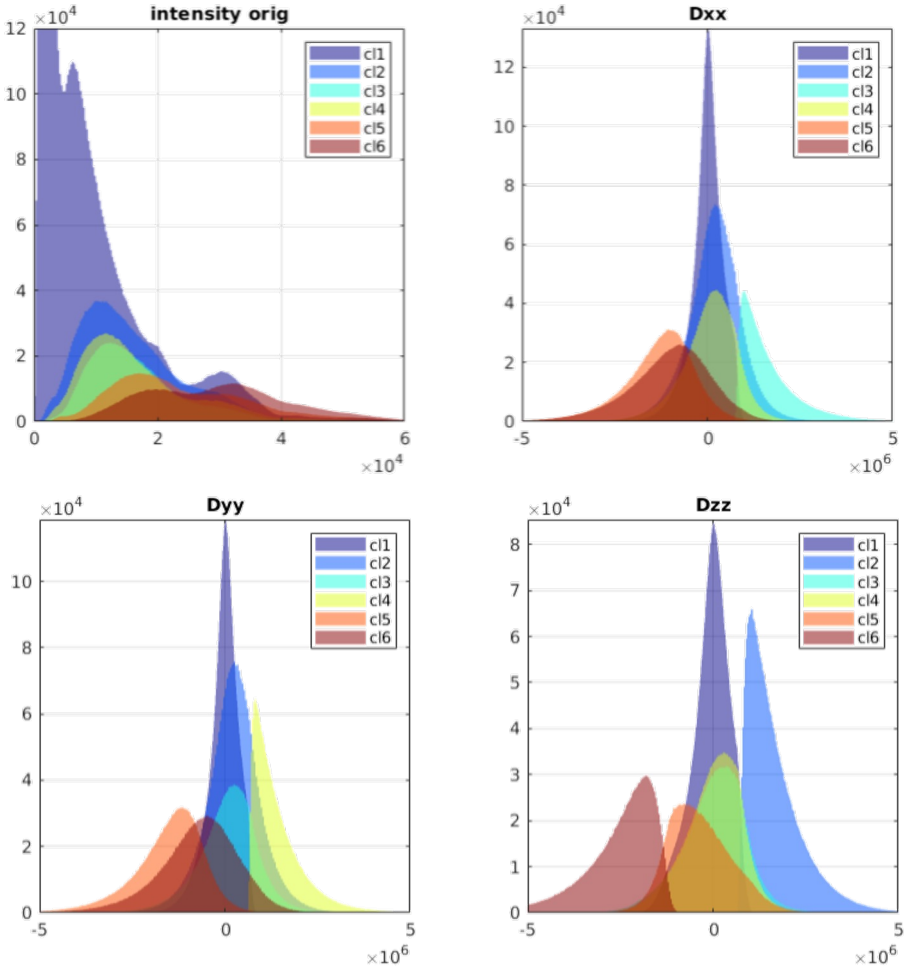
we can distinguish neuron-related classes



SENPAI: a topological informed data driven approach to neuronal reconstruction

A k-means algorithm exploiting spatial derivatives





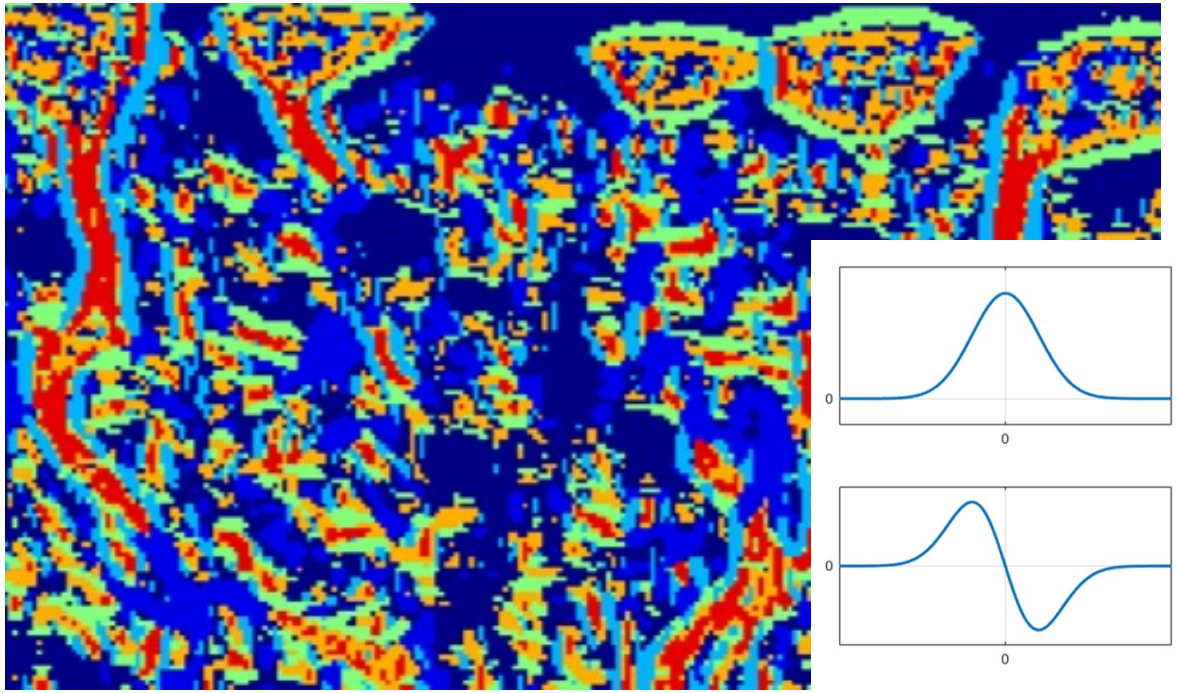
Classes are chosen according to:

- negative mean of the derivatives
- high intensity

Cauzzo S et al, Parcellation of binary segmentations in microscopy images of ex-vivo clarified neurons via morphological reconstruction and watershed transform, FENS Forum 2022

SENPAI: a topological informed data driven approach to neuronal reconstruction

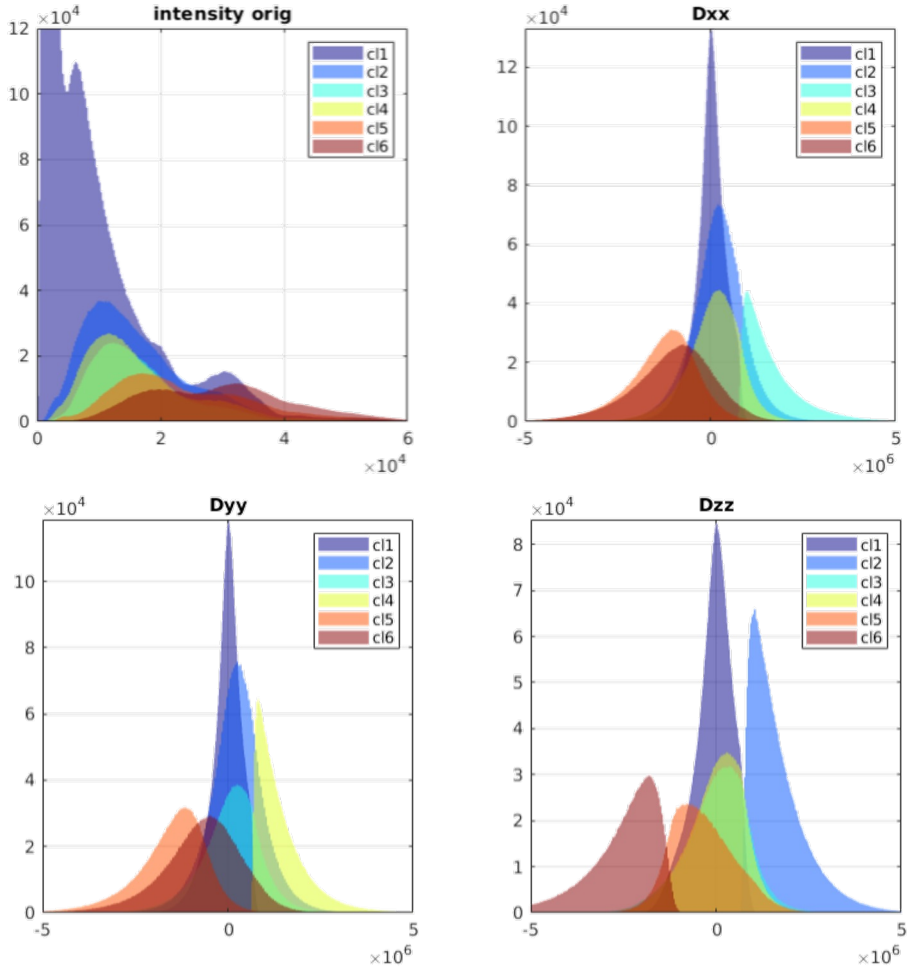
A k-means algorithm exploiting spatial derivatives



1) Profile of a dendritic branch with smooth transitions towards the background.

2) The first derivative is directionality-dependent.

3) The second derivative encodes all the inner edges of the transition with negative values.



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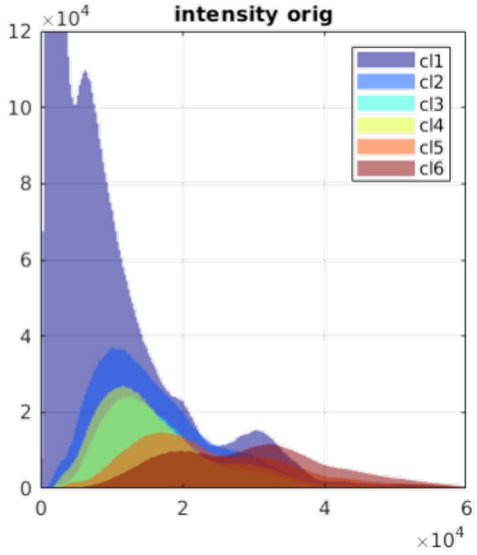
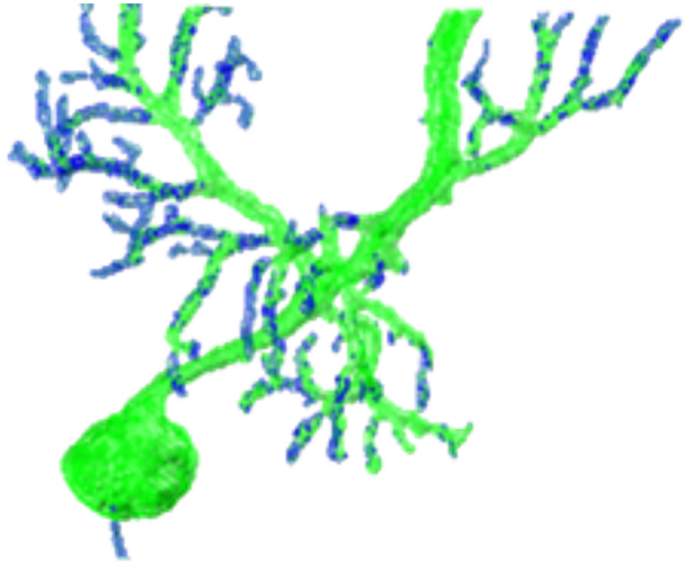
SENPAI: a topological informed data driven approach to neuronal reconstruction

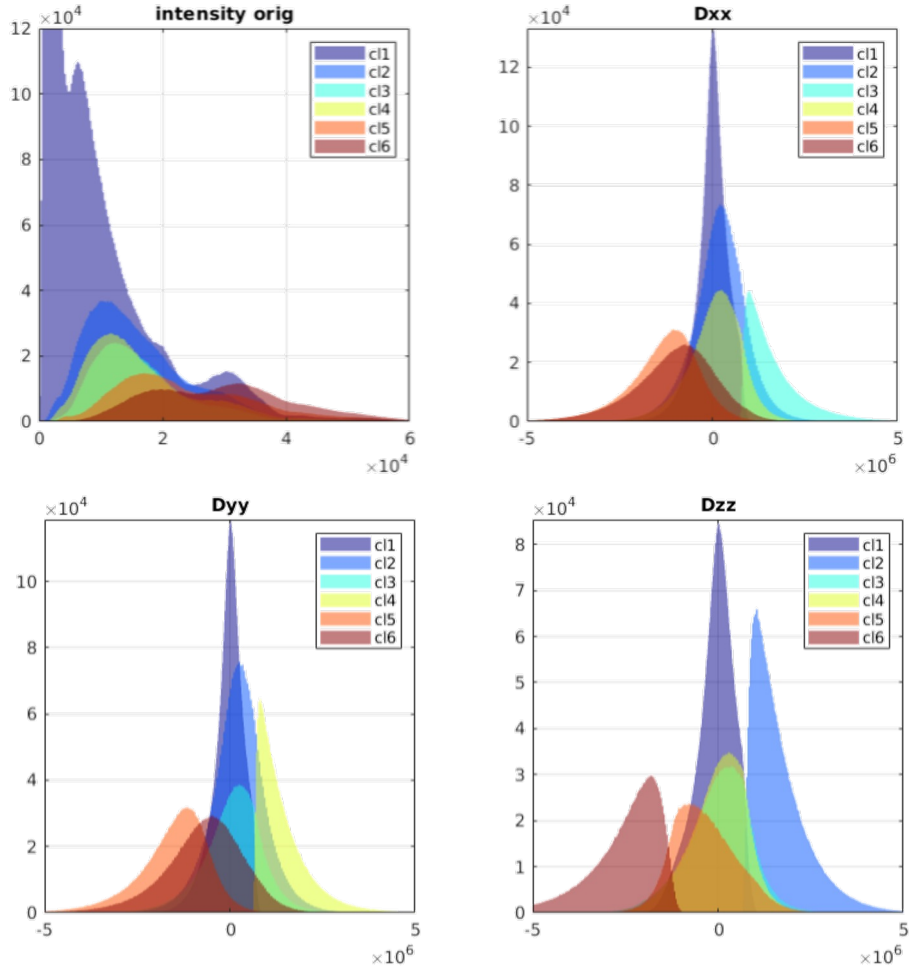
A k-means algorithm exploiting spatial derivatives



Intensity distribution of pixel classes; they overlap



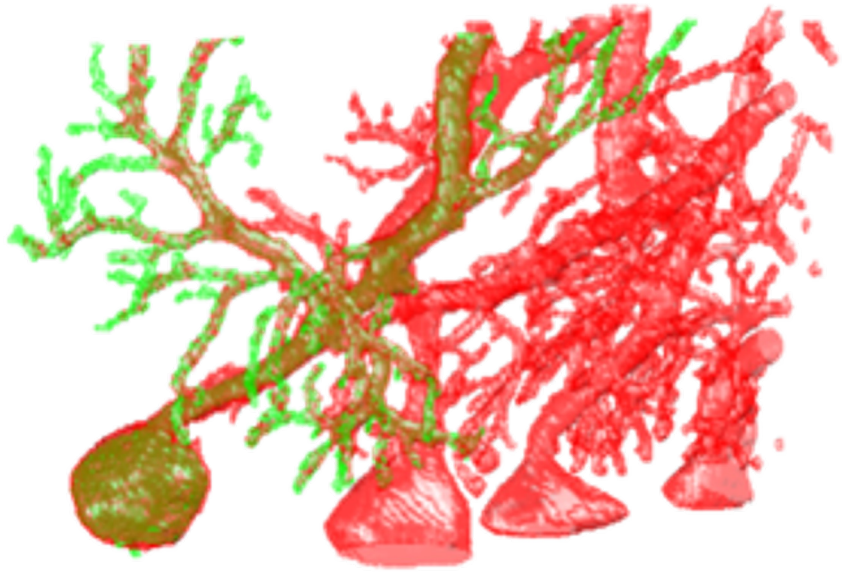
In blue low intensity pixels that were detected by adding topological info

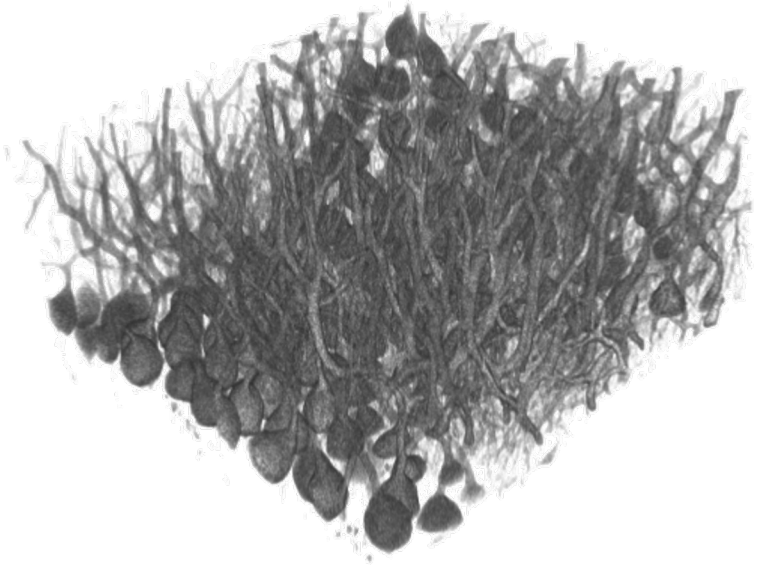


SENPAI: a topological informed data driven approach

A k-means algorithm exploiting spatial derivatives

We might fail the identification of single structures in dense images

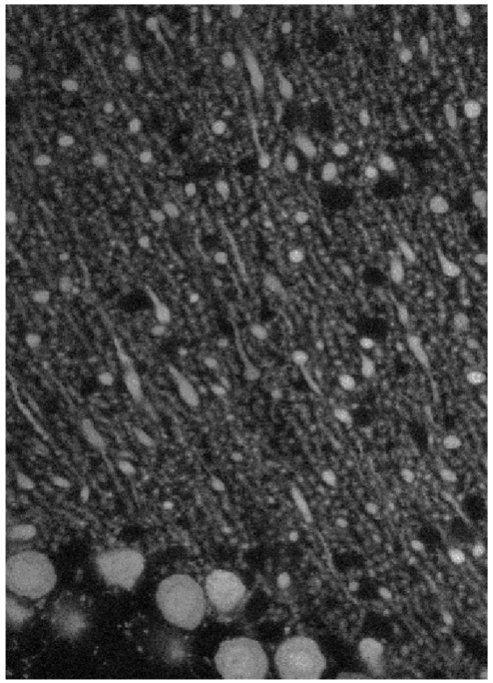




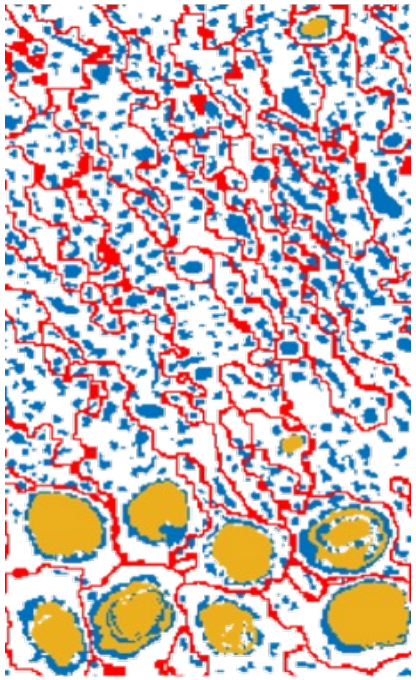
- green could not be detected using intensity
- neurons are easily merged together: single neuron identification is envisaged

SENPAI: a topological informed data driven approach

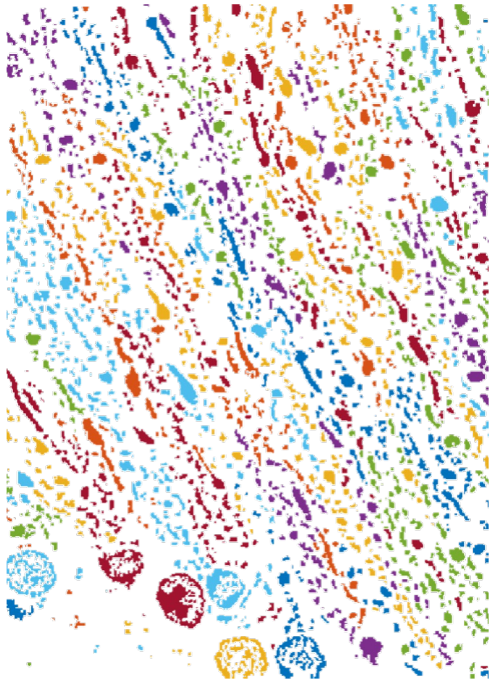
A k-means algorithm exploiting spatial derivatives… and watershed



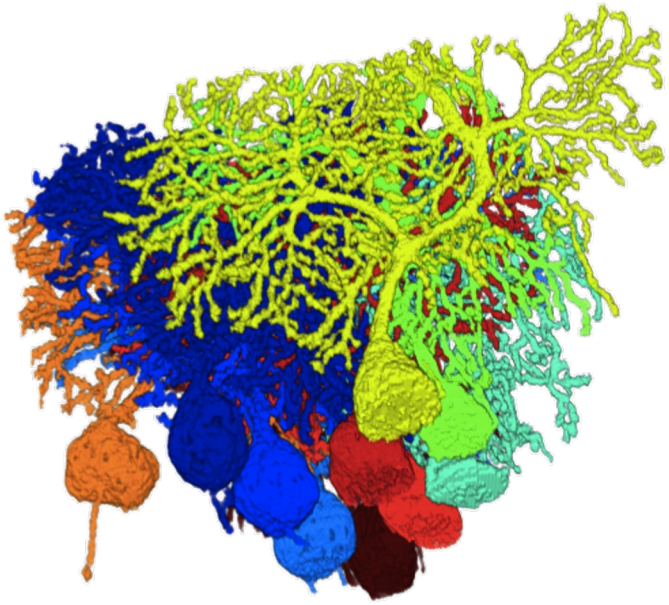
Original image
40x



Watershed on
segmented data



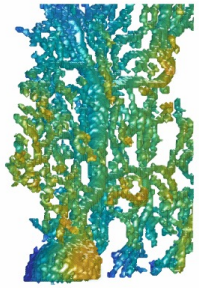
Parcellation

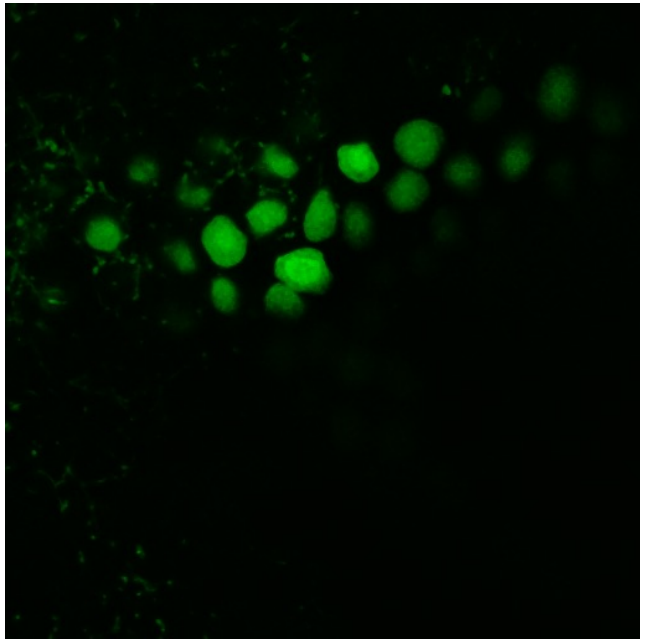


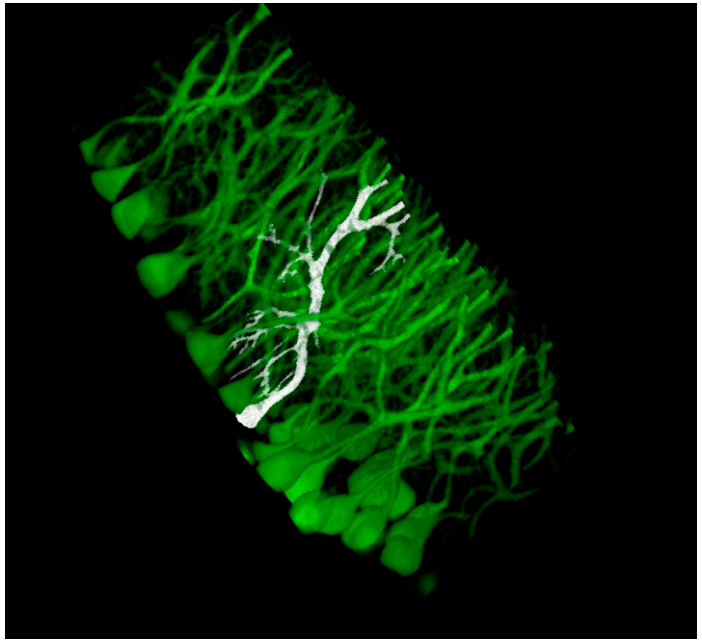
3D recon

SENPAI: a topological informed data driven approach

A k-means algorithm exploiting spatial derivatives and watershed







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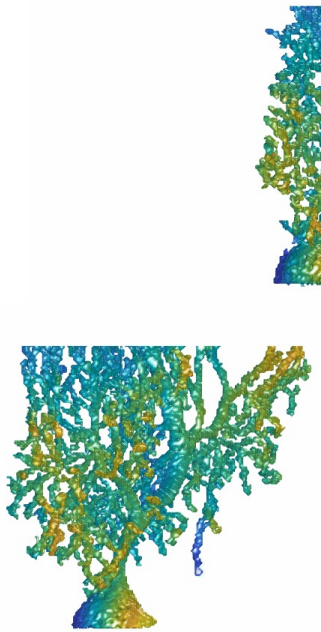
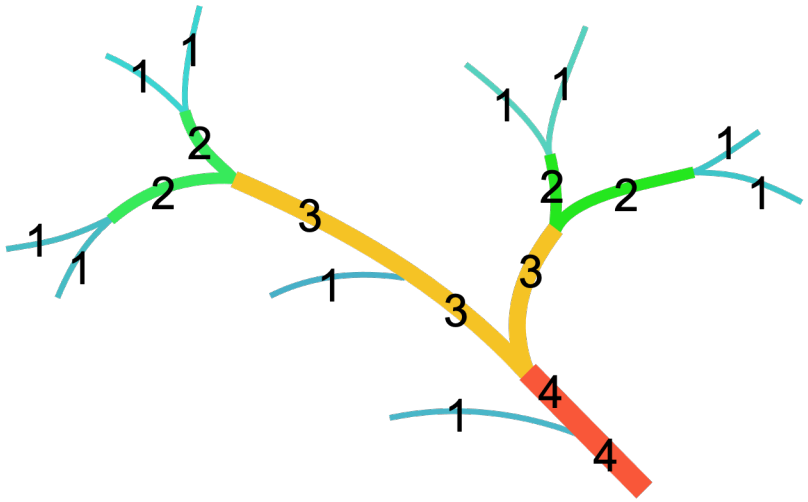
A (possible) Validation: Strahler ordering

statistical based approach



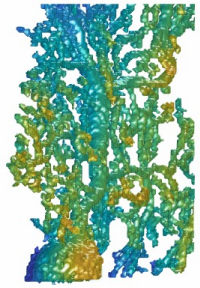
Strahler ordering is used to describe complexity branching structures

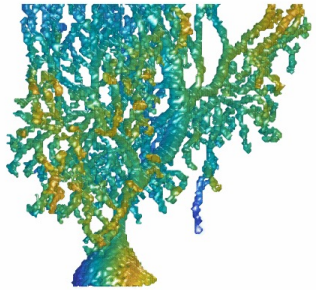


Strahler, A. N. (1957), "Quantitative analysis of watershed geomorphology", Transactions of the American Geophysical Union, 38 (6): 913–920

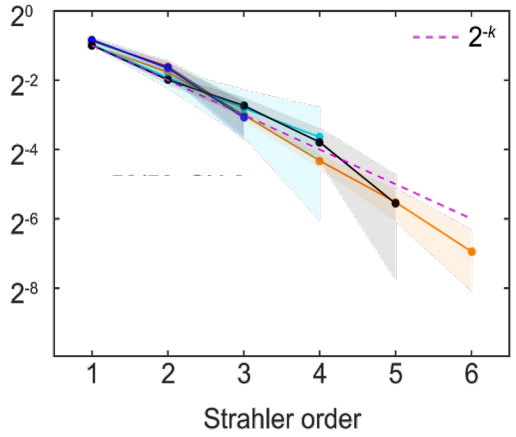
A (possible) Validation: Strahler ordering

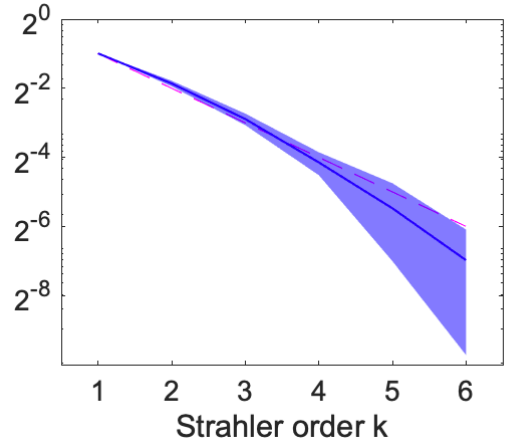
statistical based approach



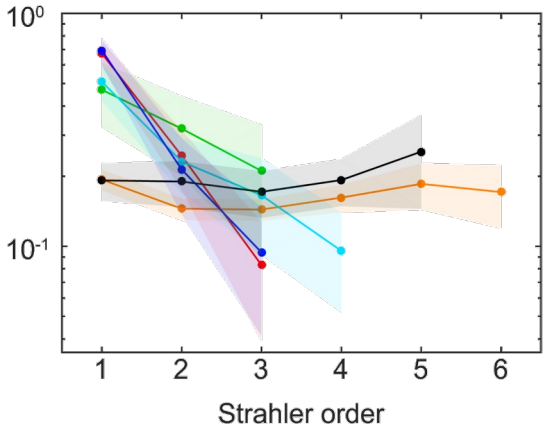


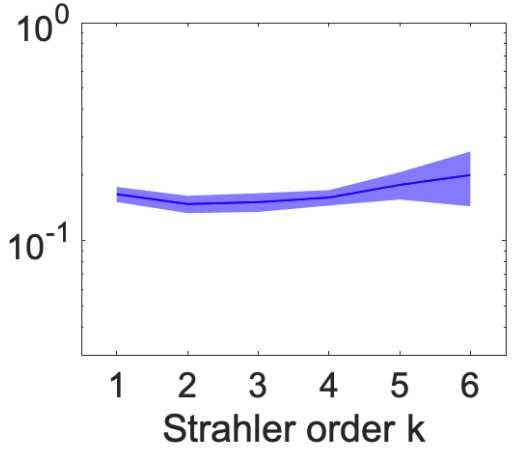
Segment number

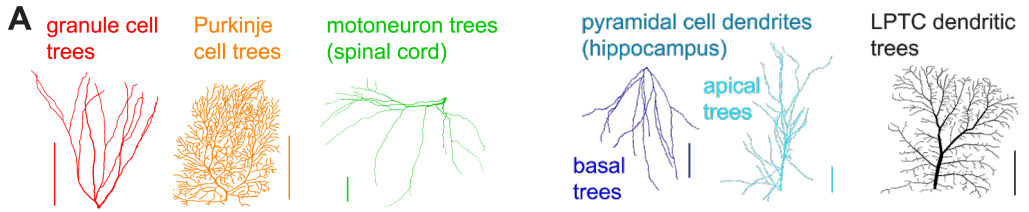




Segment length







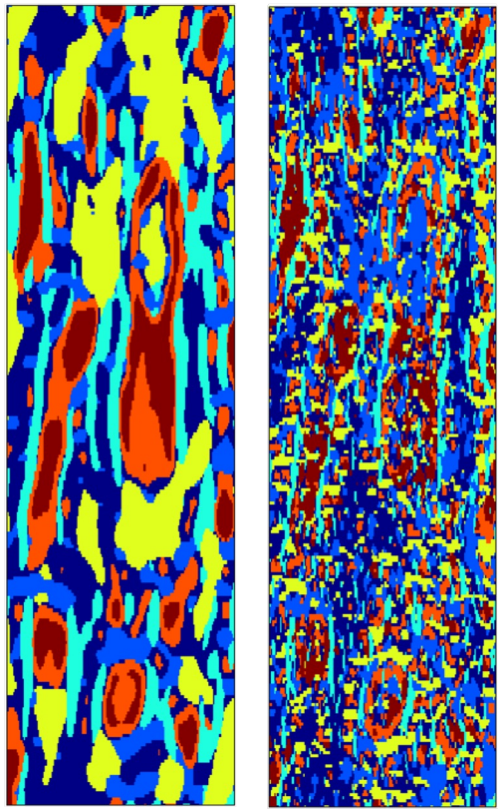
Strahler ordering-based statistics for different neural cell types, Vormberg et al., 2017

SENPAI: a topological informed data driven approach to neuronal reconstruction

Spine segmentation

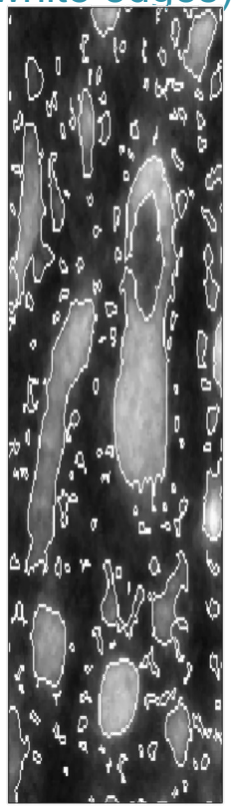
Original Image (93x)

Topology-informed k-means At different levels of smoothing



Class selection + Level Merging

Final result (segmentation in white edges)



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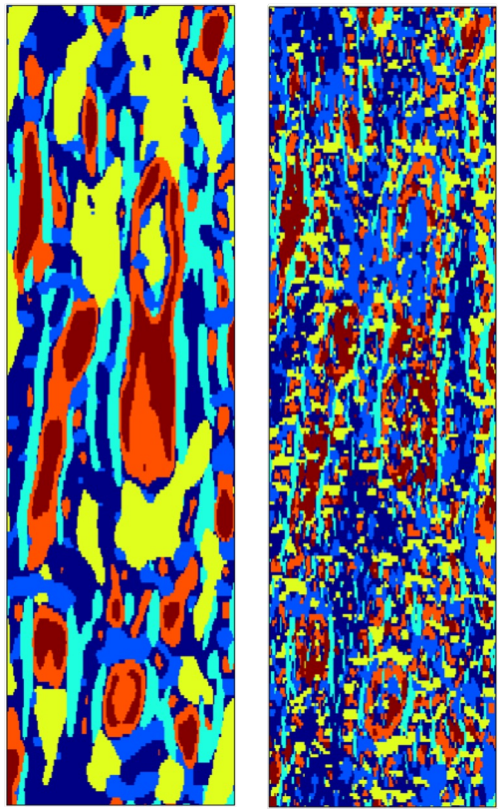
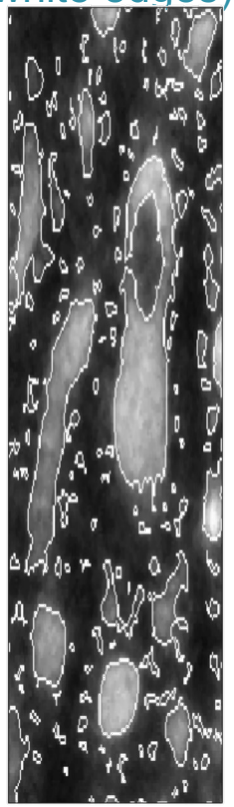
Original Image (93x)

Topology-informed k-means At different levels of smoothing



Class selection + Level Merging

Final result (segmentation in white edges)



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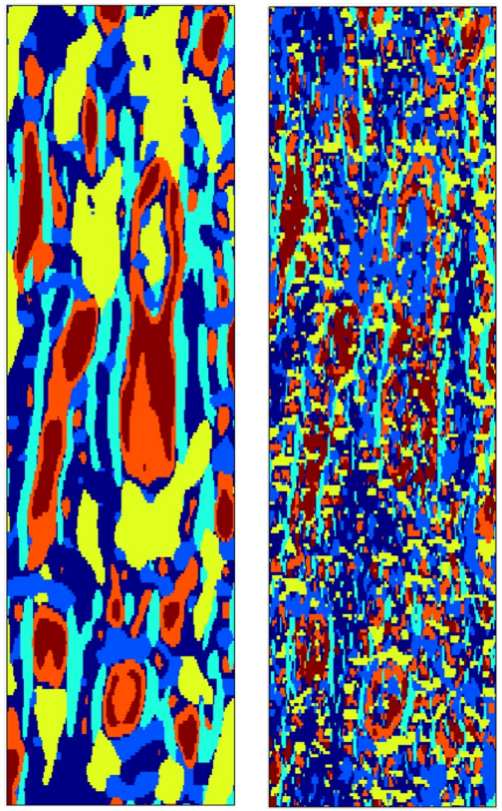
Spine segmentation

Original Image (93x)

Topology-informed k-means At different levels of smoothing



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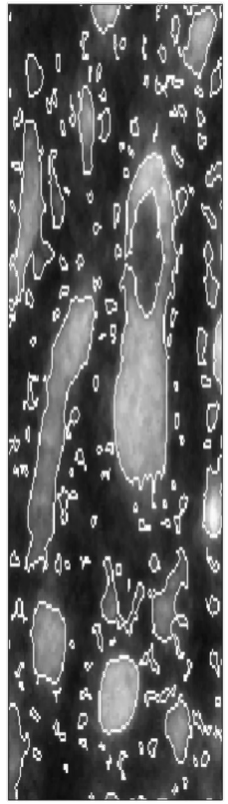
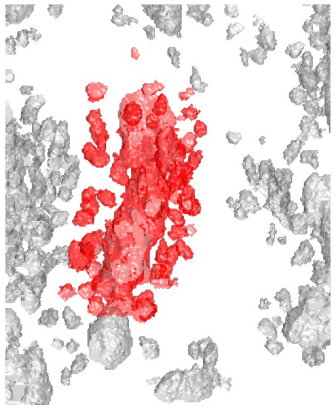


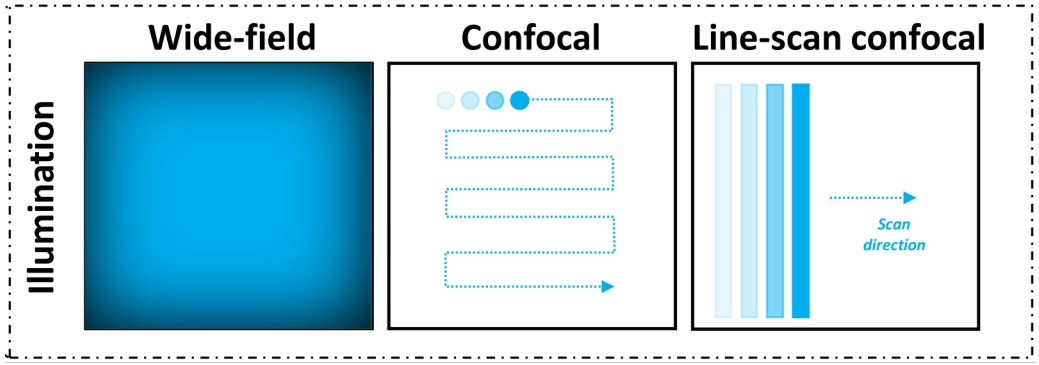
SENPAI

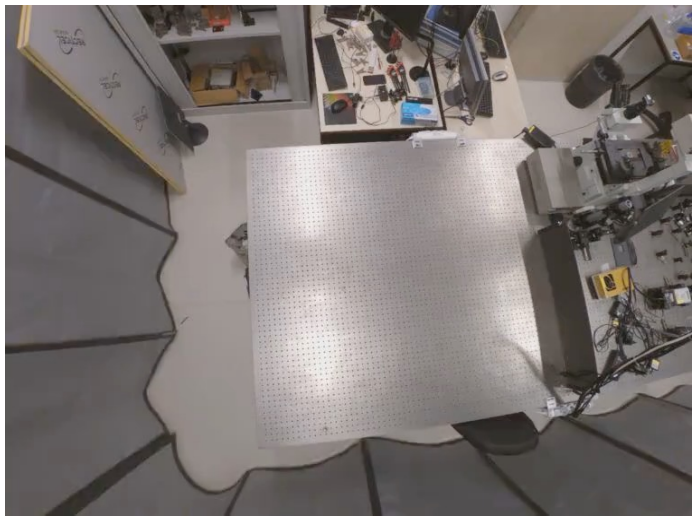


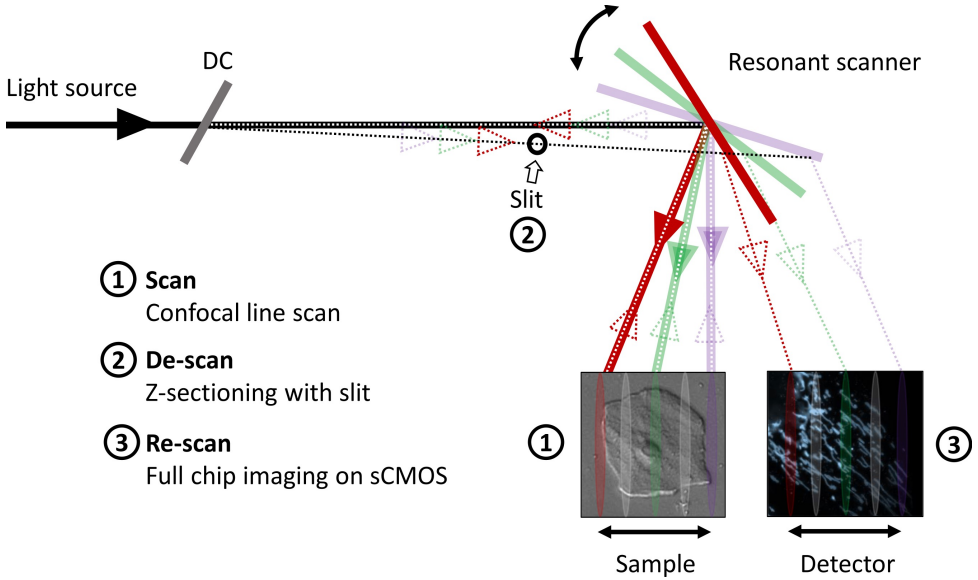
Manual

Triscan

Fast tissue imaging / SMLM using a line-scan confocal







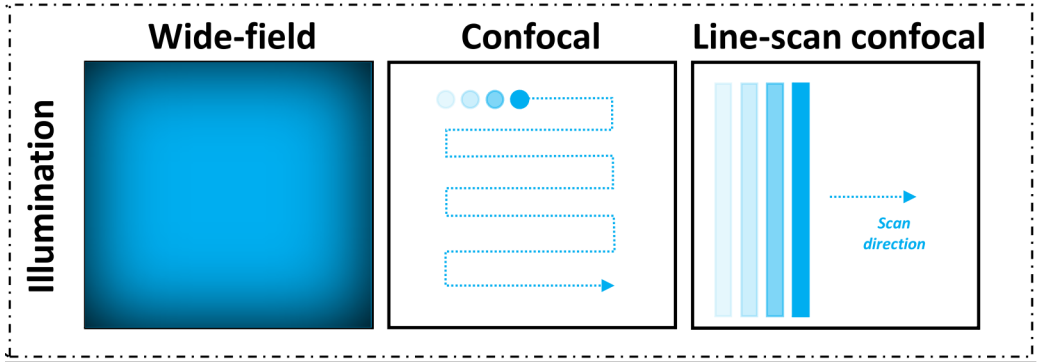
Prototype has been realized
Optimization ongoing

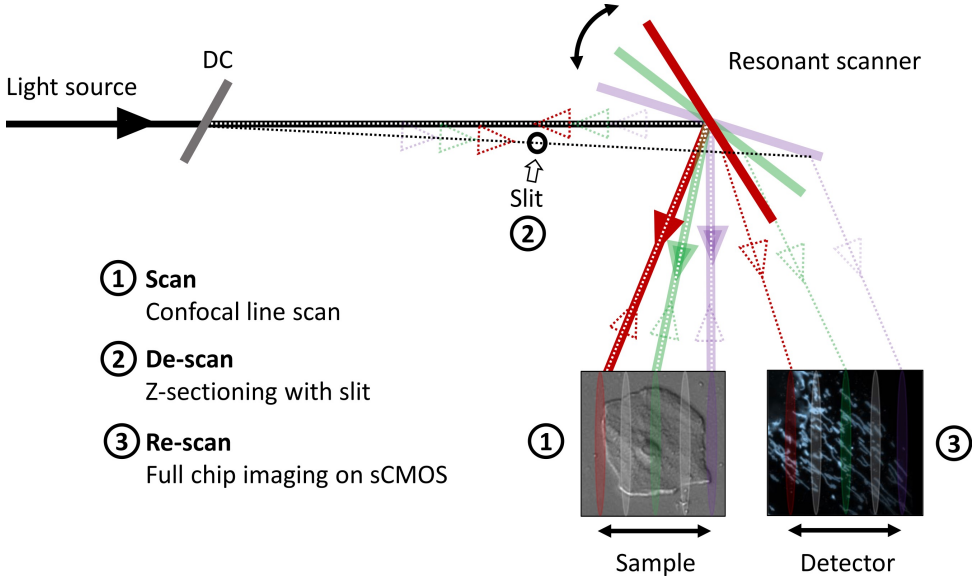
Expected performance:

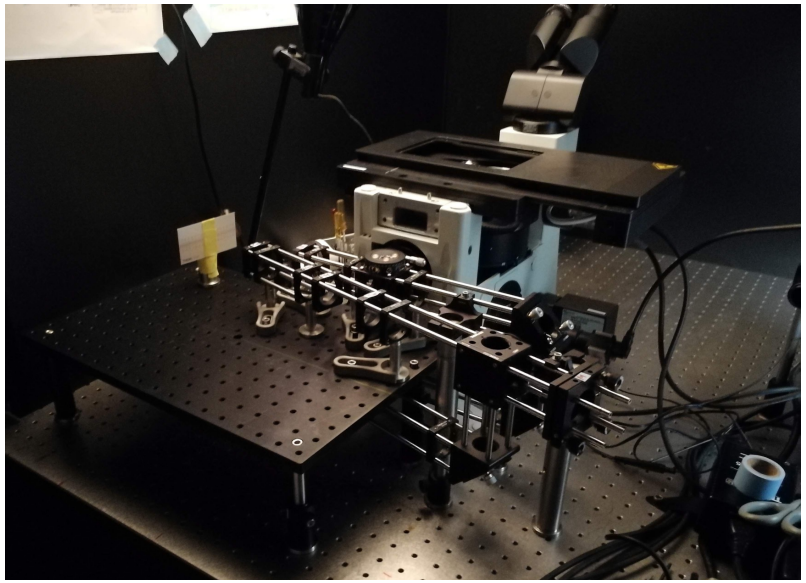
- Faster than classical confocal
- Similar resolution in *xy*
- Slightly reduced sectioning in *z*
- Single-molecule sensitive

Triscan

Fast tissue imaging / SMLM using a line-scan confocal







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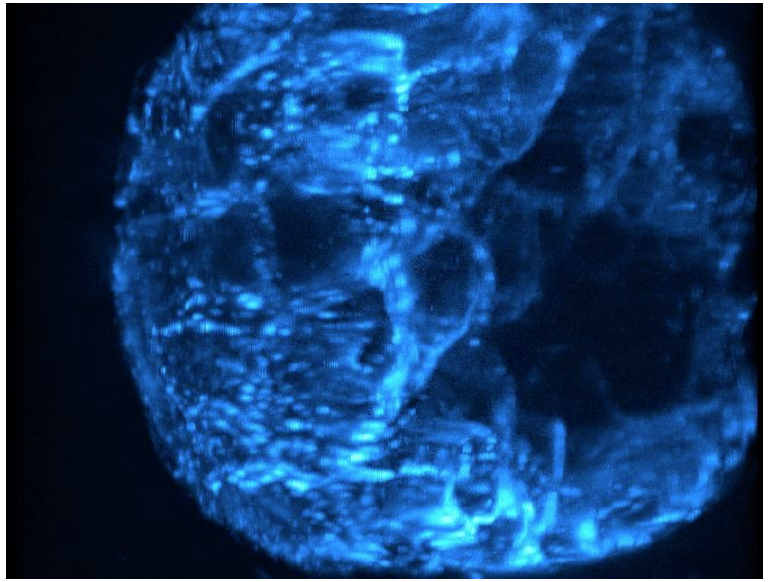
Triscan

Fast tissue imaging / SMLM using a line-scan confocal



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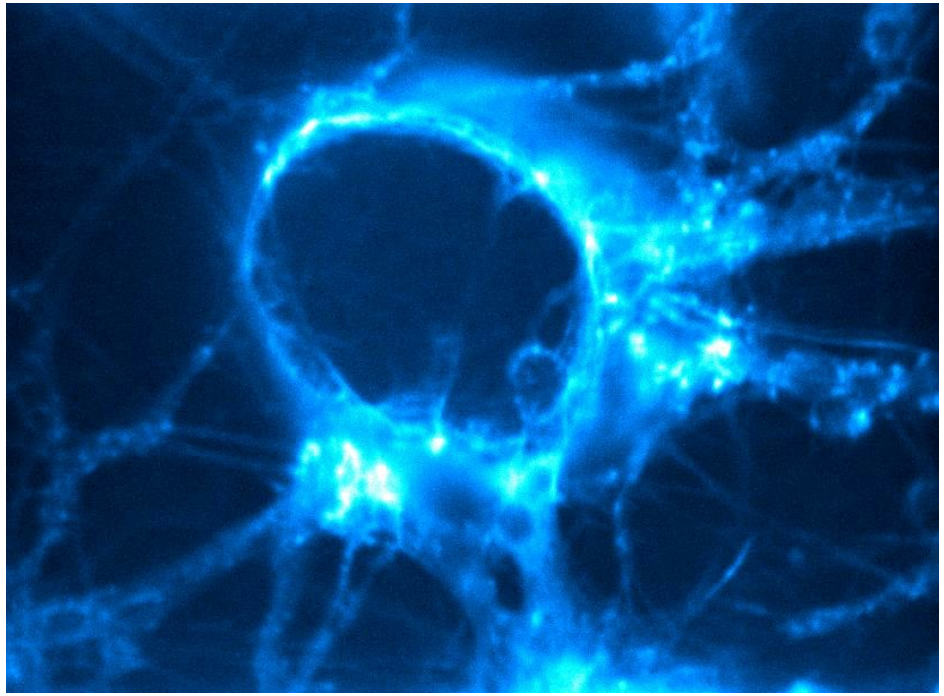
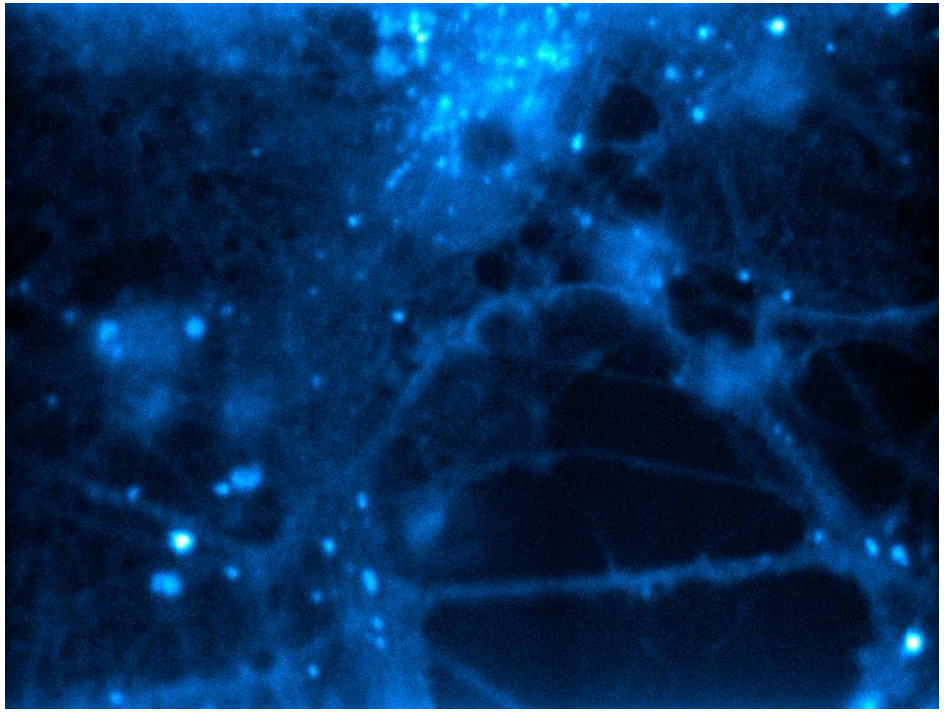
Triscan

Fast tissue imaging / SMLM using a line-scan confocal



Without Triscan



Triscan

Hippocampal neurons: actin labelled with phalloidin Alexa488

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SENSEI & EBRAINS

- At this time we are not using any EBRAINS tool

- A lot of time was spent to reach SENSEI objectives

  Limited time/people for learning the use of other tools/services

- SENSEI was not aware about the possible added value of using EBRAINS services/tools, taking into account the corresponding learning curve

SENSEI outcomes

- Images obtained with different imaging modalities
  -
- Algorithms for neuron segmentation
  -
  -
- Triscan
  -

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SENSEI outcomes

- Images obtained with different imaging modalities
  - Some images were shared via Zenodo
- Algorithms for neuron segmentation
  - SmRG (model based segmentation, available on GitHub)
  - SENPAI (soon available)
- Triscan
  - prototype level

Callara A.L., Magliaro C., Ahluwalia A., Vanello N. A Smart Region-Growing Algorithm for Single-Neuron Segmentation From Confocal and 2-Photon Datasets (2020) Frontiers in Neuroinformatics, 14, art. no. 9

SENSEI next steps

- Acquire human samples
  - Surgery resection from S. Anne Hospital, Paris
- Apply the segmentation algorithm to spine morphology/density estimation
- Develop multiscale data integration (e.g. 40x and 63x)
- Share the algorithms and test with other datasets
  - use data in the EBRAINS repository
  - candidate our algorithms for becoming “EBRAINS tools” (i.e. used by EBRAINS users and more…)
- Strengthen the collaborations and creating new ones
  - exploit EBRAINS collaboratory environment
  - use EBRAINS tools for setting up a protocol for registering the images/reconstruction on Atlases

Human Brain Project

EBRAINS

Thank you

www.humanbrainproject.eu

www.ebrains.eu

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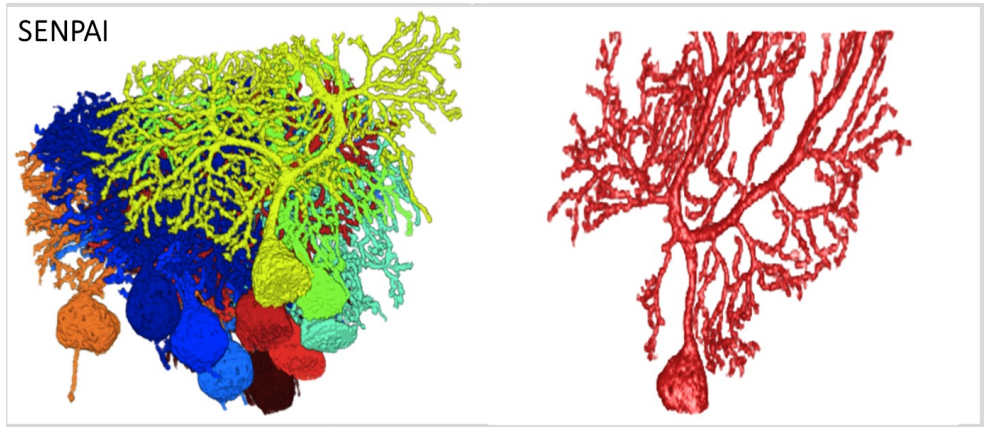
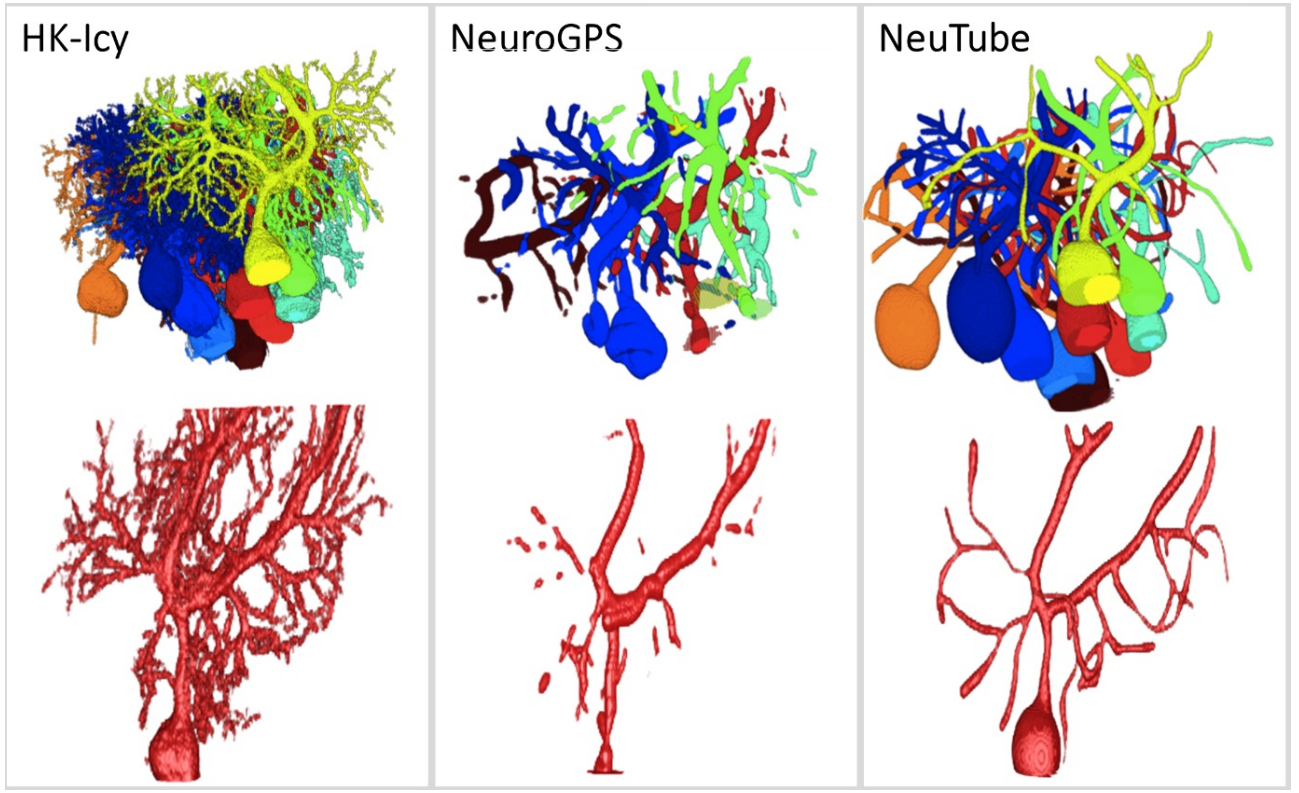
Algorithm Comparison





SENPAI: a topological informed data driven approach to neuronal reconstruction

Algorithm Comparison