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
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
Dendritic spine morphology is associated to learning & ageing


SENSEI Team

SEGmentation of Neurons using Standard and supEr-resolution microscopy

Lydia Danglot

Peter Dedecker
Labeling Strategy to decipher neural shape within tissue slices

Tissue Clarification

Electrodes

In plane resolution [μm]

Penetration depth [mm]

Confocal

Multi-photon

Light sheet

CLARITY

STED

Electron
Acquisitions with different microscopy modalities

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

- Spinning disk: 20x 63x
- Confocal Leica/3D sted: 93x
- Confocal Leica: 63x/25x
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

If we use spatial derivatives

The analysis with Hessian allows to describe different spatial distributions

Intensity distribution of pixel classes; they overlap
SENPAI: a topological informed data driven approach to neuronal reconstruction

A k-means algorithm exploiting spatial derivatives

If we use spatial derivatives (2nd order)...

we can distinguish neuron-related classes

Intensity distribution of pixel classes; they overlap
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

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

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
A (possible) Validation: Strahler ordering
statistical based approach

Strahler ordering is used to describe complexity branching structures


Kilom691, CC BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0>, via Wikimedia Commons
A (possible) Validation: Strahler ordering
statistical based approach

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)
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)
SENPAI: a topological informed data driven approach to neuronal reconstruction

Spine segmentation

Original Image (93x)  Topology-informed k-means
At different levels of smoothing

Final result
(segmentation in white edges)

Class selection + Level Merging

SENPAI  Manual
Triscan
Fast tissue imaging / SMLM using a line-scan confocal

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

Prototype has been realized
Optimization ongoing
Triscan
Fast tissue imaging / SMLM using a line-scan confocal

Expected performance:
- Faster than classical confocal
- Similar resolution in $xy$
- Slightly reduced sectioning in $z$
- Single-molecule sensitive

Prototype has been realized
Optimization ongoing
Triscan
Fast tissue imaging / SMLM using a line-scan confocal
Triscan
Fast tissue imaging / SMLM using a line-scan confocal

Hippocampal neurons: actin labelled with phalloidin Alexa488
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
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

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
Thank you

www.humanbrainproject.eu  www.ebrains.eu
Algorithm Comparison

SENPAI

HK-Icy

NeuroGPS

NeuTube
SENPAI: a topological informed data driven approach to neuronal reconstruction

Algorithm Comparison

<table>
<thead>
<tr>
<th></th>
<th>SENPAI</th>
<th>HK-Icy</th>
<th>Ilastik</th>
<th>Manual</th>
</tr>
</thead>
</table>