AUTOMATED ANALYSIS OF NEURONAL MORPHOLOGY: DETECTION, MODELING AND RECONSTRUCTION

Thesis Defense

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NUS
National University of Singapore

Overview

- Introduction

- S1. ***Detection*** of tubular structure networks
- S2. ***Modeling*** single neurite morphology
- S3. ***Reconstruction*** of neuronal trees

- Conclusion

Why neuron morphology analysis?

- **New era of high content, high resolution imaging**
- **Structure** determines **functions**

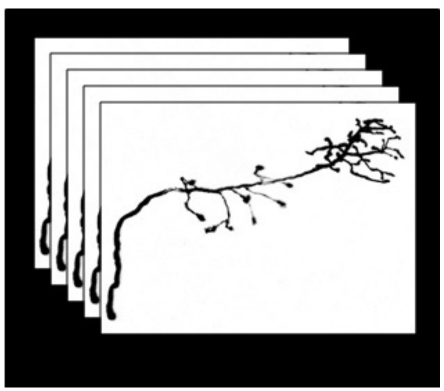
Application

- **Brain mapping /connectomics**
- **Brain atlas/ neuromics**

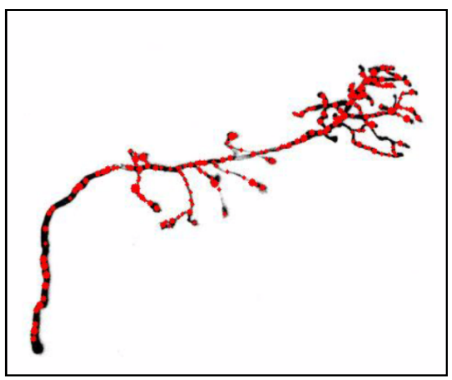
	Neurons	**Synapses**	
Caenorhabditis elegans	302	~5000	15 yrs of man hours
Drosophila melanogaster	$\sim 10^5$	$\sim 10^7$	800 TB (SSTEM data)
Homo sapiens	$\sim 8.5 \times 10^{10}$	$\sim 10^{15}$	>> 800 TB

Thesis Objective

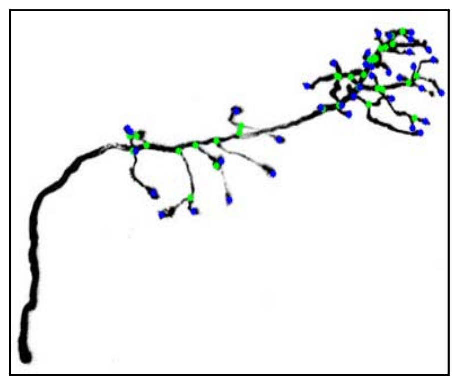
- To automate ***detection***, ***modeling*** and ***reconstruction*** of neuronal morphology from 3D light microscopy (**LM**) image stacks.



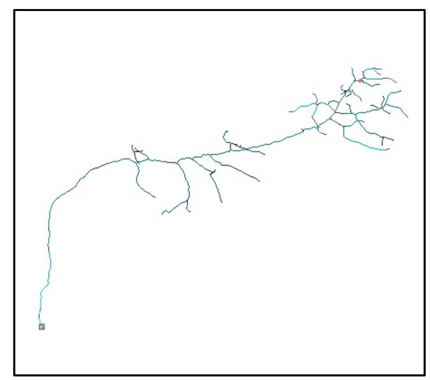
3D image stack



Detection



Modeling



Reconstruction

Workflow of Neuron Morphology Analysis

Image Acquisition
- Fixing, sectioning, staining
- Imaging [**LM**]

Pre-processing
- Stitching/Mosaicking
- Registration

Neuron Image Database

Digital Reconstruction
- Detection, segmentation
- Automated tracing

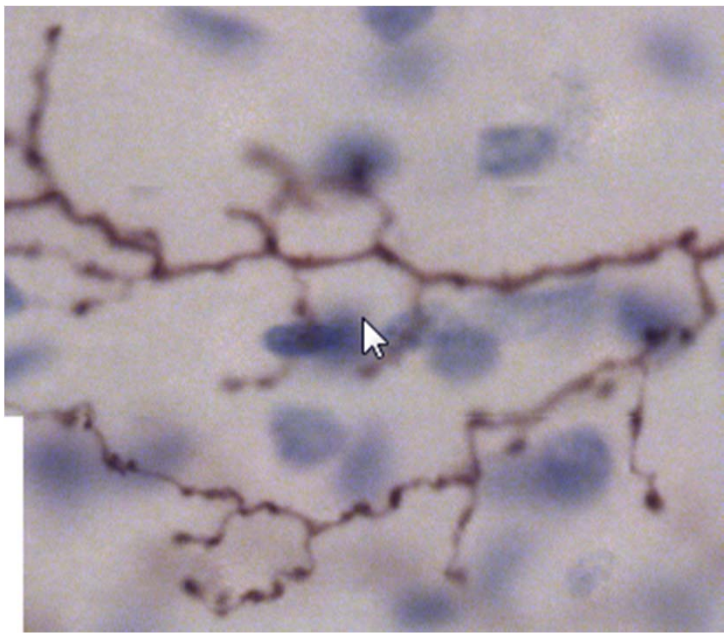
S1,S3

Modeling & Analysis
- Classification, Simulation
- Statistical inference

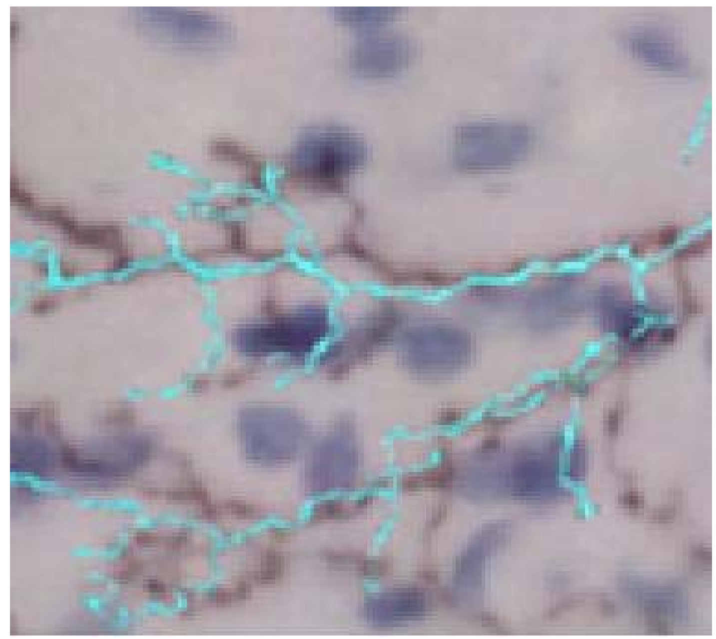
S1, S2

3D Neuronal Data

Neuronal morphology in 3D image stack



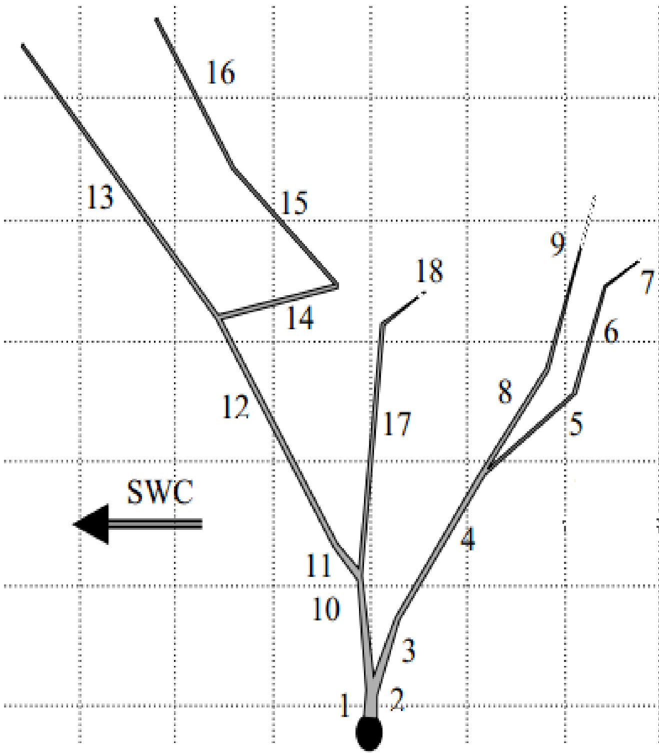
Manual reconstruction projected on 1 slice



Digital Reconstruction

Example *3D* reconstruction in SWC format for tree structure quantification as MST

ID	T	X	Y	Z	R	C
1	1	−1.5	−8.2	1.3	3.8	−1
2	3	0.8	4.2	1.4	2.3	1
3	3	21.1	41.1	1.4	1.3	2
4	3	89.2	115.1	−5.3	1.1	3
5	3	153.6	163.5	3.0	0.6	4
6	3	184.9	218.1	−2.3	0.5	5
7	3	220.9	238.7	−13.7	0.3	6
8	3	159.9	183.9	−23.6	1.1	4
9	3	211.9	270.9	−35.2	0.7	8
10	3	0.6	58.1	−2.6	1.1	2
11	3	−0.5	75.9	−18.6	1.1	10
12	3	−40.5	180.0	−55.3	1.0	11
13	3	−156.4	281.8	−65.9	0.8	12
14	3	0.3	186.8	−7.0	0.8	12
15	3	−51.5	235.7	−27.0	0.8	14
16	3	−85.2	297.6	−41.5	0.5	15
17	3	75.3	198.3	−54.6	0.8	10
18	3	130.6	228.6	−81.3	0.5	17

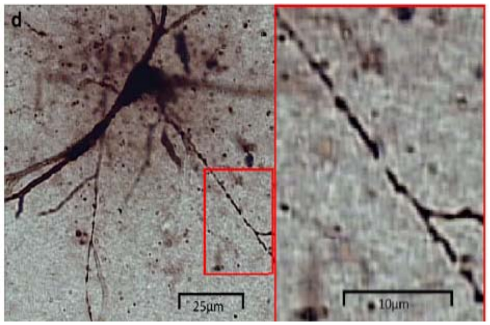


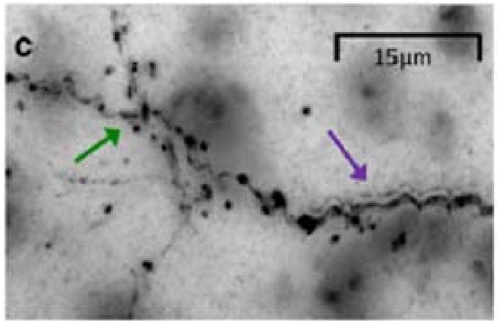
Digitization : Easier to archive, exchange, analyze

Figure adapted from : Ascoli, G. A., J. L. Krichmar, et al. (2001). "Generation, description and storage of dendritic morphology data." Philos Trans R Soc Lond B Biol Sci 356(1412): 1131-1145.

Challenges

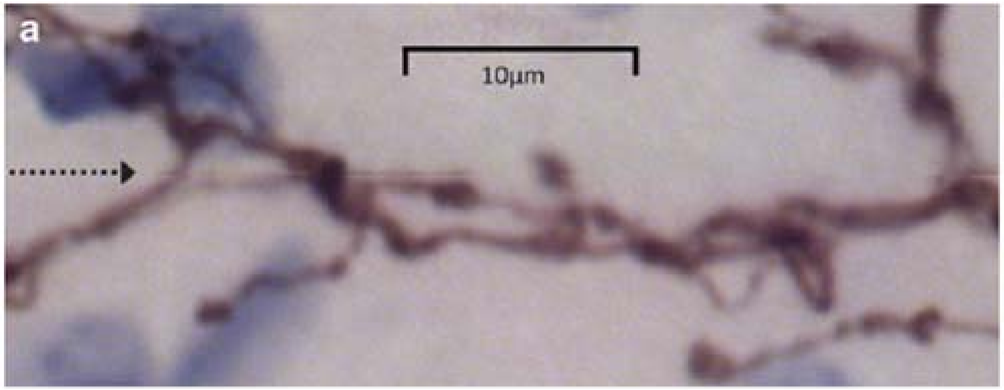
Branch gaps and discontinuities





Shadows around branches mistaken as parallel processes

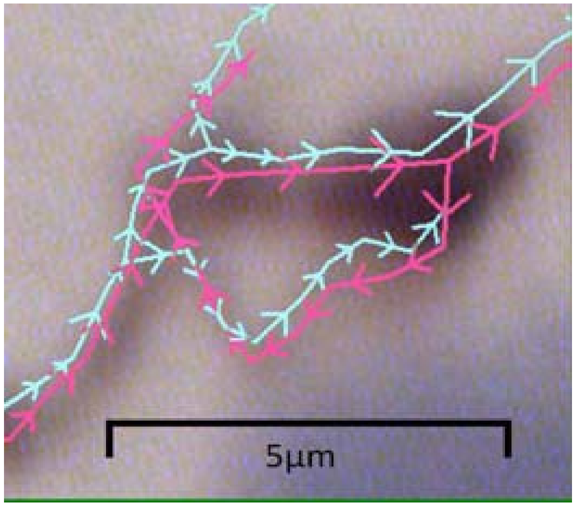
Tile stitching artifacts: Structured noise misinterpreted as neuronal structure



Inhomogeneous intensity, fuzzy edges

Brown, K. M., G. Barrionuevo, et al. (2011). "The DIADEM data sets: representative light microscopy images of neuronal morphology to advance automation of digital reconstructions.“ Neuroinformatics **9**(2-3): 143-157

Challenges



Variability in GOLD STANDARD
manual reconstruction (GSR)

Brown, K. M., G. Barrionuevo, et al. (2011). "The DIADEM data sets: representative light microscopy images of neuronal morphology to advance automation of digital reconstructions.“ Neuroinformatics **9**(2-3): 143-157

Data Sets Used

DIADEM challenge database

Neuron class	Imaging modality	Animal	
Olfactory Projection Fibres (OPF)	2-channel confocal microscopy	Drosophila	S1,S2,S3
Cerebellar Climbing Fibres (CCF)	Transmitted Light Brightfield	Rat	S1,S2,S3
Neocortical Layer 1 Axons (NL)	2-photon lasers scanning microscopy *in vivo*	Mouse	S1

Related Work

3D stack of microscopy image slices

Global

Segmentation;
Centre-line detection

Trace optimization-
branch merger,
loop elimination

Local

Detection of seed points

Connecting seeds
- Active contours
- Iterative model matching

MST; Identification
of critical points

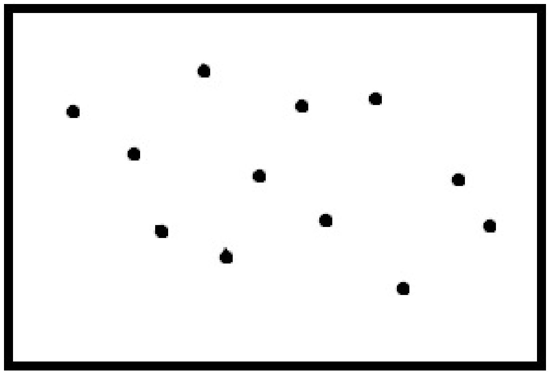
Related Work

Global algorithms	Local algorithms
•'Neuronal Reconstruction'	•'Neurite Tracing'
•Computationally intensive; high memory requirements	• Less intensive computation; dependence on seeds
•Results in less cases of disconnected components	• Can interpolate only through small intensity gaps
•Exploits more image evidence using optimization like MCMC, ACO etc	• localized search; tracing errors result in large topological perturbations
Lu et. al. PLoS ONE, 2009	Chothani et. al. Neuroinformatics 2011
Peng et. al. BMC Bioinformatics 2011	Chromanska et. al. Frontiers in Neural Circuits, 2012
Gonzalez et. al. Neuroinformatics 2011	Bas et. al. Neuroinformatics 2011

Overview

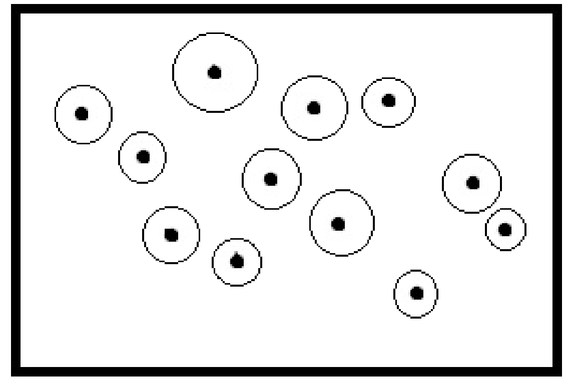
- Introduction
- S1. Detection of tubular structure networks
  - Marked point process methodology
  - Evolution of configurations
  - Energy model for neurons
  - Results
- S2. Modeling single neurite morphology
- S3. Reconstruction of neuronal trees
- Summary

Marked Point/Object Process



configuration of points on $[0, X] \times [0, Y]$

Point Process (PP)
(modeling spatial distribution of data)



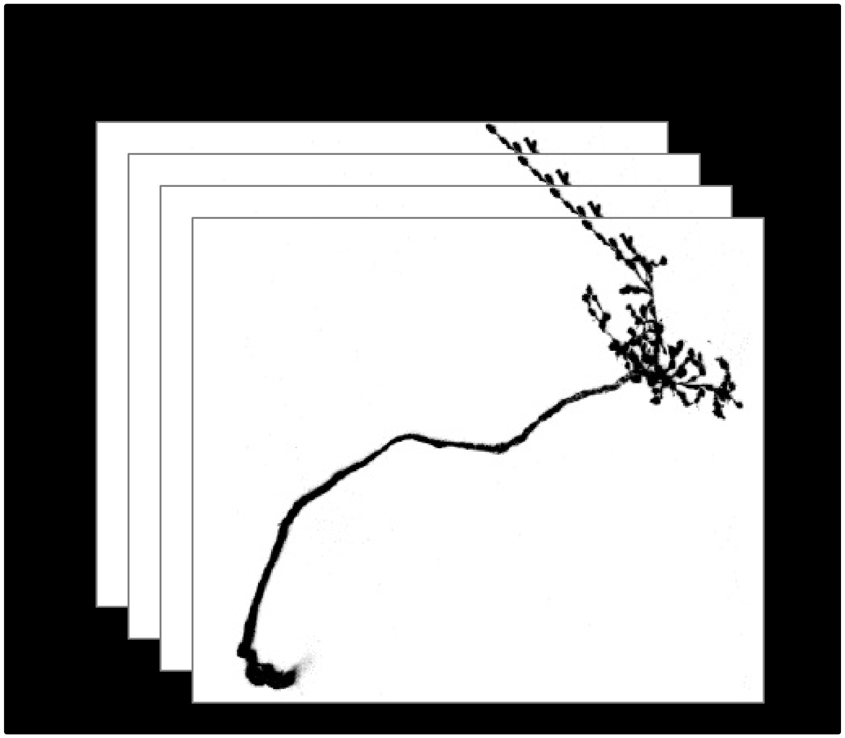
configuration of objects with centres on $[0, X] \times [0, Y]$ and marks from $[r_{min}, r_{max}]$

Marked Point Process (MPP) *(modeling spatial data + geometry of objects)*

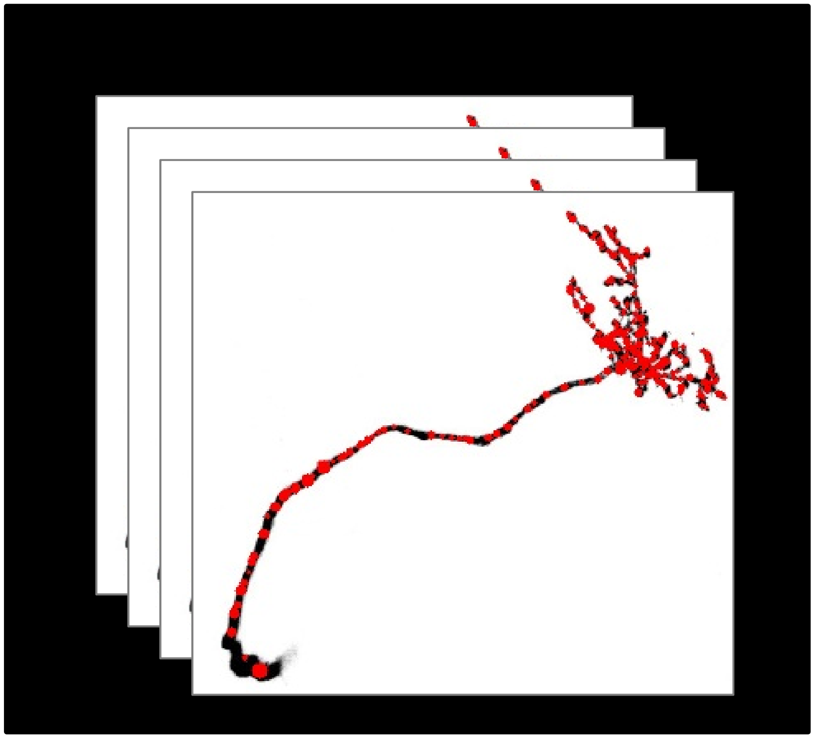
Configuration: A countable, unordered set of objects.

MPP models find an *optimal configuration of objects* from observed data i.e. image

Objective (S1)



Input - 3D stack of image slices
Olfactory Projection Fibres - OP4



Output - Extracted neuronal morphology by optimal MPP object configuration

[Basu et. al. MICCAI'13]

3D MPP model

Aim: unsupervised 3D neuronal network extraction

Object: sphere $\omega_i = (\underset{\text{centre}}{x_i}, \underset{\text{radius}}{m_i})$

centre *radius*

Object space: $[0, X_{max}] \times [0, Y_{max}] \times [0, Z_{max}] \times [r_{min}, r_{max}]$

Configuration: $\gamma_n \in \Omega_n, \gamma_n = \{\omega_1, \ldots, \omega_n\}$

$$\Omega = \cup_{n=0}^{\infty} \Omega_n$$

Gibbs Field framework

The (posterior) probability distribution on the configuration space has the Gibbs form:

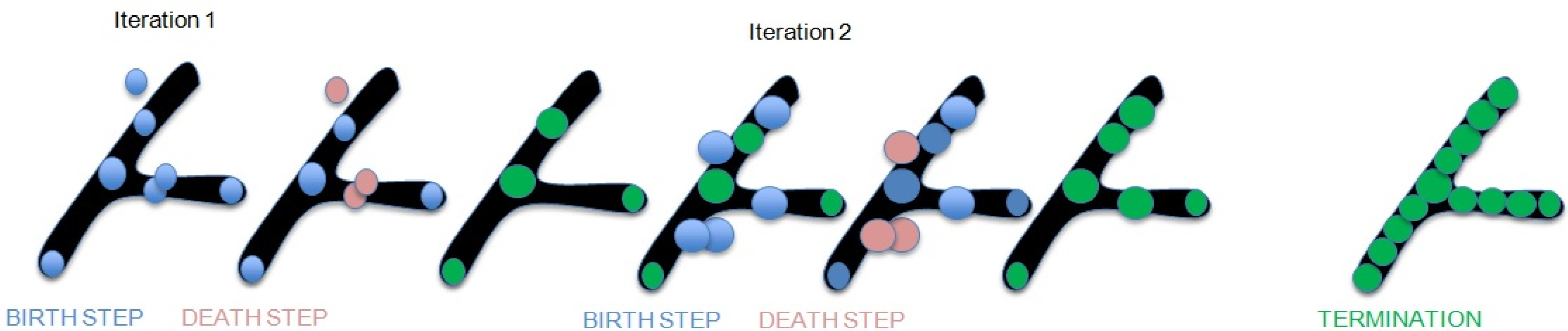
$$p(\gamma|\theta) = k_\beta \exp[-\beta U(\gamma, \theta)]$$

Energy: $U(\gamma) = w_d \underbrace{U_d(\gamma)}_{\text{Data Energy}} + \underbrace{U_p(\gamma)}_{\text{Prior Energy}}$

Optimization* : discrete time Markov chain **Multiple Birth and Death** dynamics embedded in **Simulated Annealing**

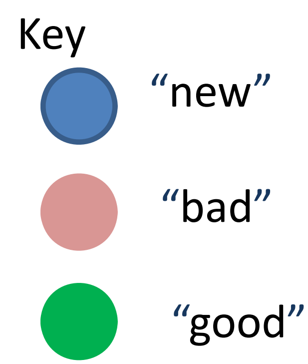
* X. Descombes, R. Minlos, and E. Zhizhina. Object extraction using a stochastic birth-and-death dynamics in continuum. Journal of Mathematical Imaging and Vision, 33(3):347-359, 2009.

Iterative Optimization Scheme

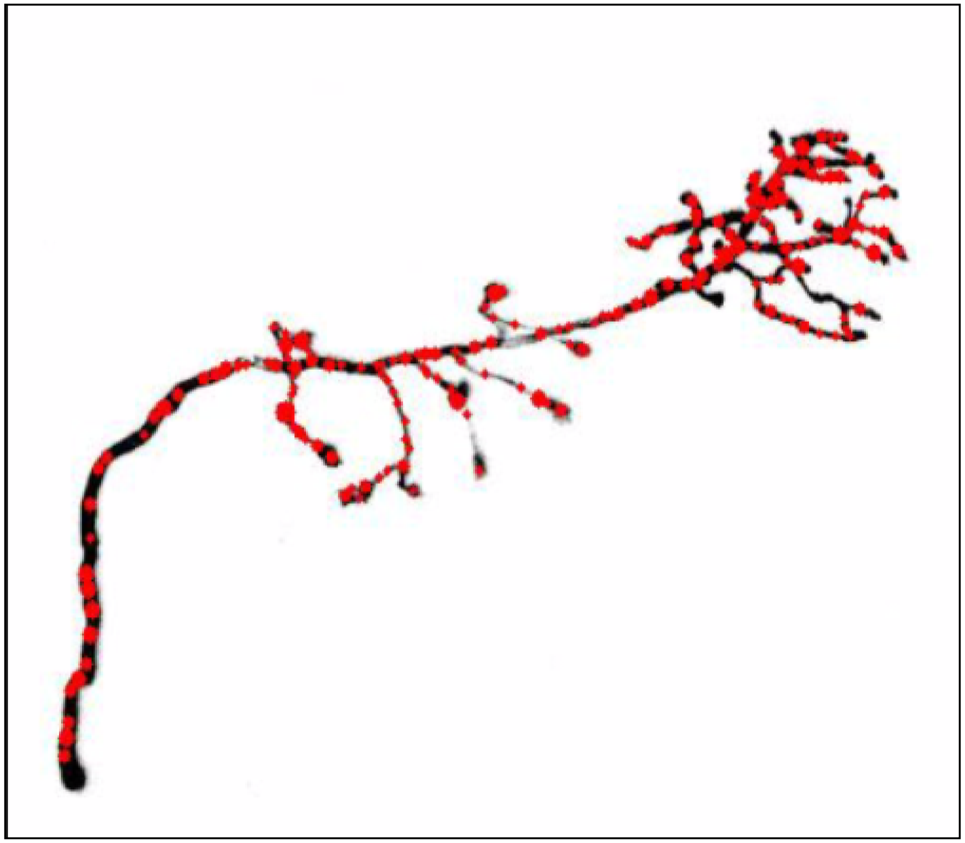


Advantages

- Unsupervised extraction
- Macro-object interaction
- Incorporation of geometric and interaction constraints



Evolution of Configuration



Video

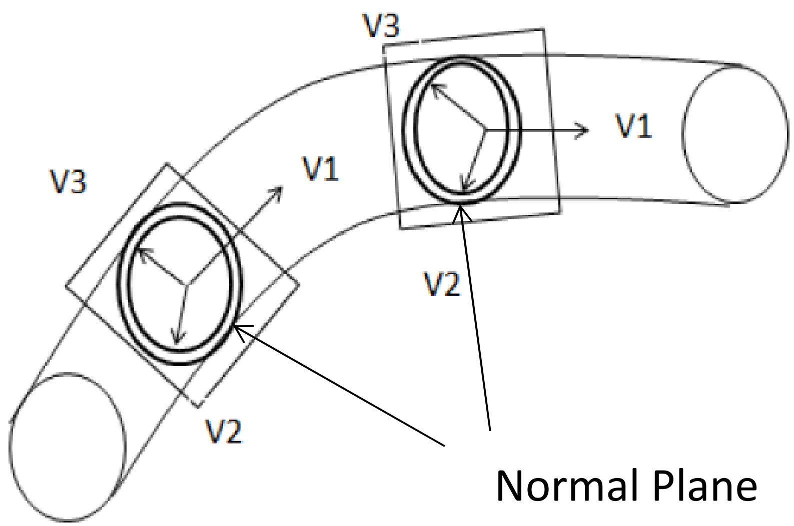
Energy function: Data Energy

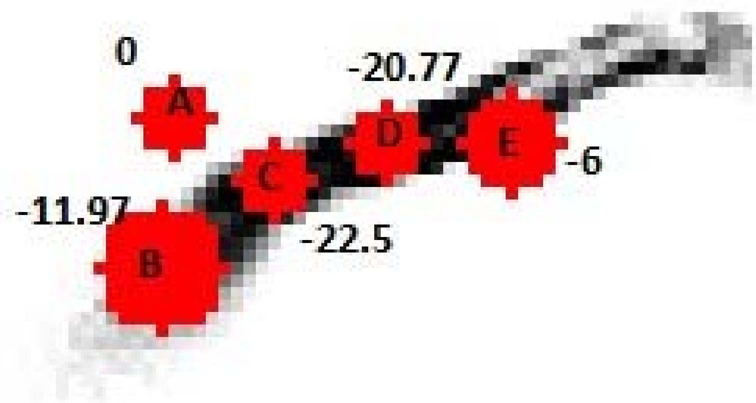
$$U(\gamma) = \boxed{U_d(\gamma)} + U_i(\gamma) + U_c(\gamma)$$

$$U_d(\gamma) = \sum_{\omega_i \epsilon \gamma} U_d(\omega_i)$$

Data term:

- Checks fit to data
- Measures neuriteness of voxels





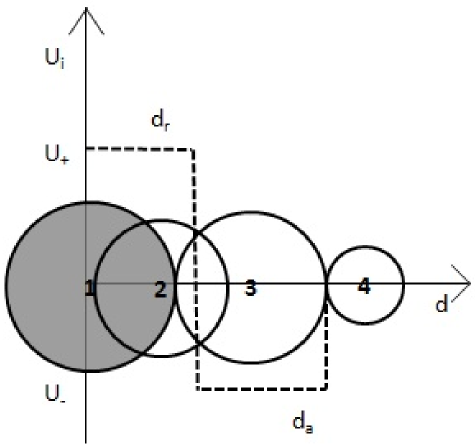
Reference : Pock, T., Janko, C., Beichel, R., Bischof, H.: Multiscale medialness for robust segmentation of 3d tubular structures. In: Proceedings of the Computer Vision Winter Workshop. (2005) 93–102

Energy function: Prior I

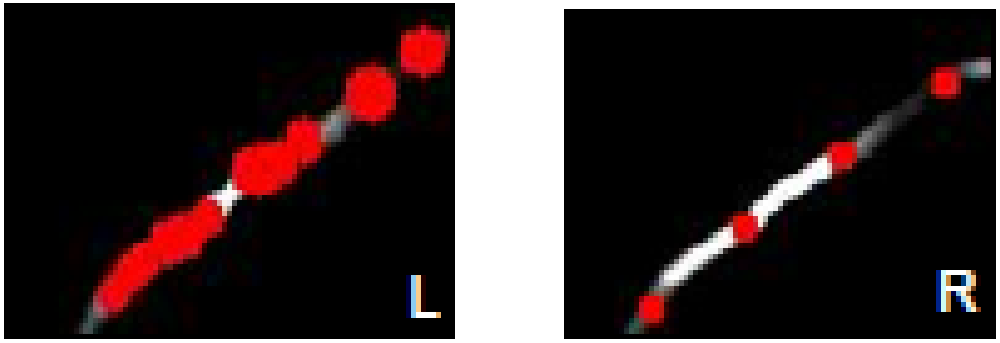
$$U(\gamma) = U_d(\gamma) + \boxed{U_i(\gamma)} + U_c(\gamma)$$

$$U_i(\gamma) = \sum_{\substack{\omega_i,\omega_j \in \gamma; \\ |\omega_i - \omega_j| < d_a}} U_i(\omega_i, \omega_j)$$

Pair-wise interaction constraints:
- Prevents overlap
- Favors connectedness



d_r: distance repulsion
d_a: distance attraction
U_+: positive potential
U-: negative potential



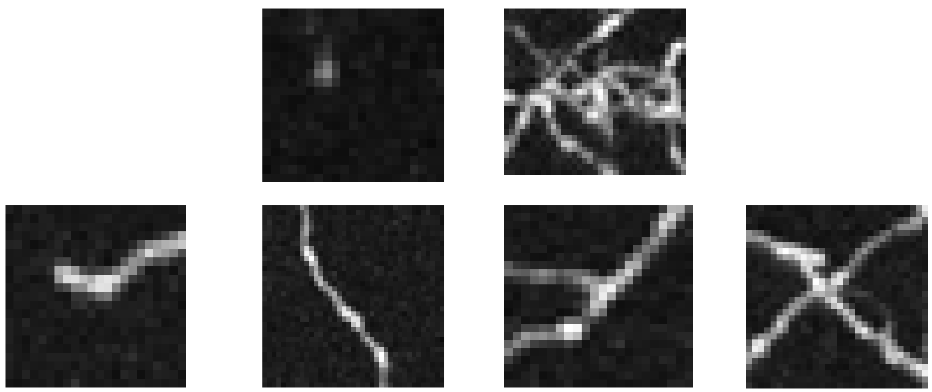
L:Considering radiometric properties
R: radiometry + interaction constraints

Energy function: Prior II

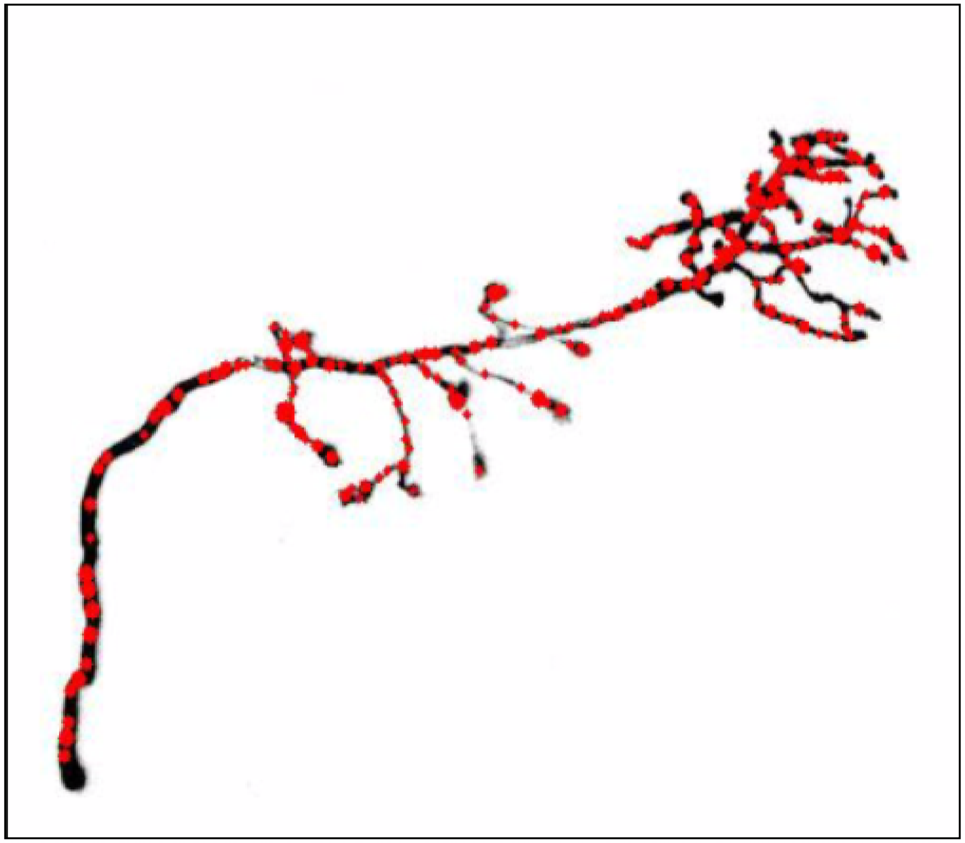
$$U(\gamma) = U_d(\gamma) + U_i(\gamma) + \boxed{U_c(\gamma)}$$

$$U_c(\gamma) = \sum_{\omega_i \in \gamma} U_c(\omega_i)$$

Multi-object interaction:
•favors or penalizes special configurations.

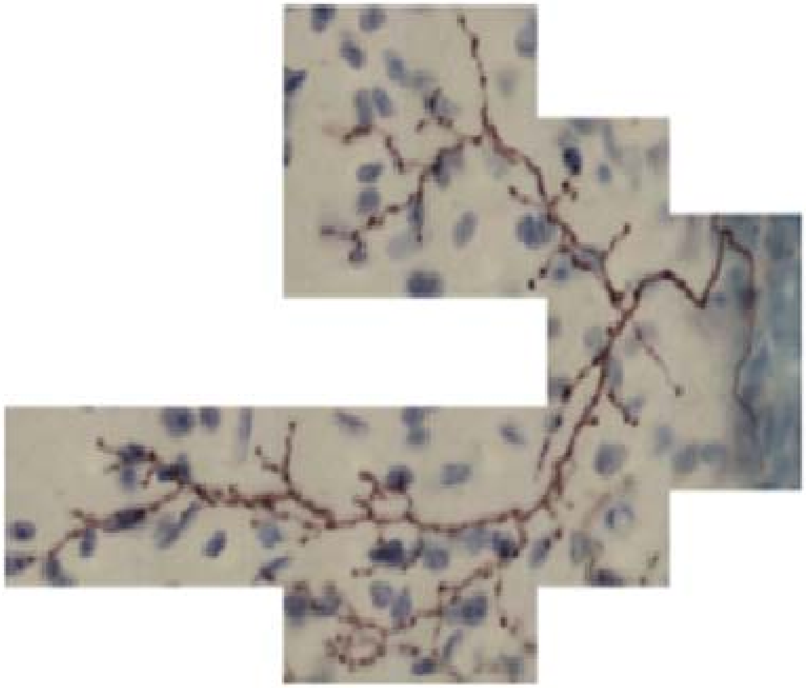


Results (S1)

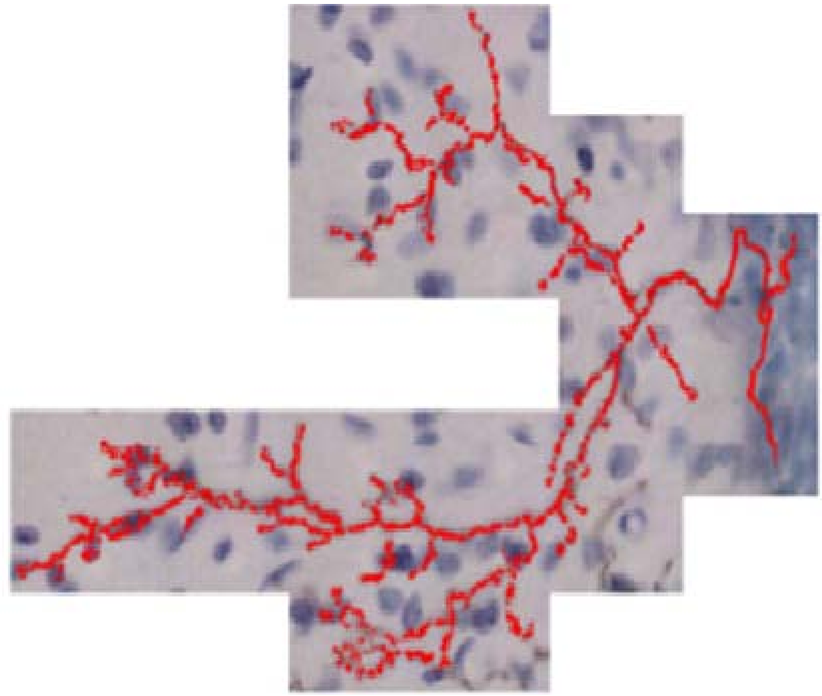


Olfactory Projection Fibres (OP1)
Confocal microscopy (video)

Results (S1)



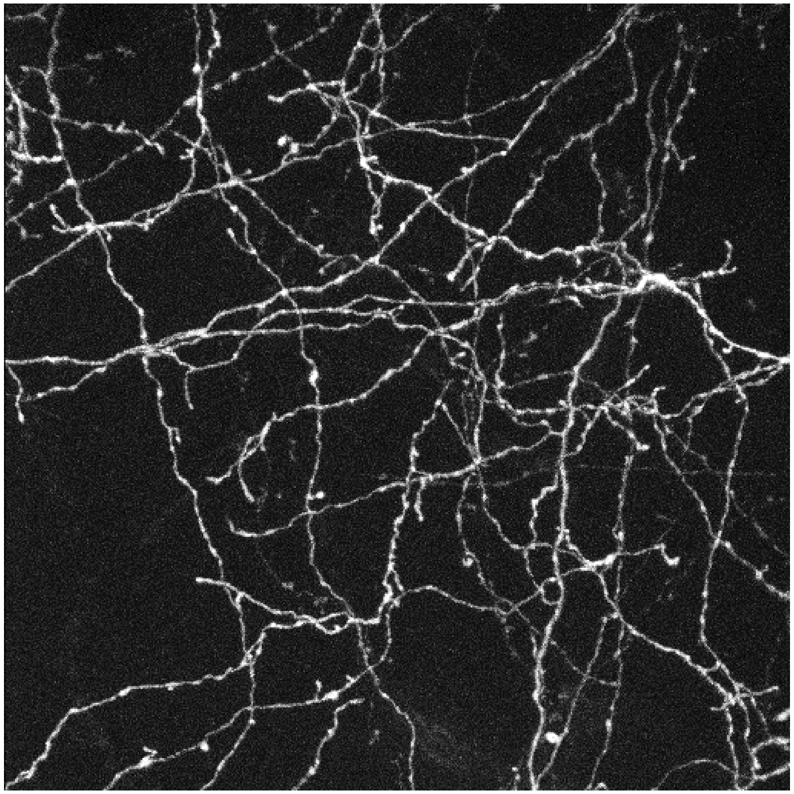
Maximum Intensity Projection



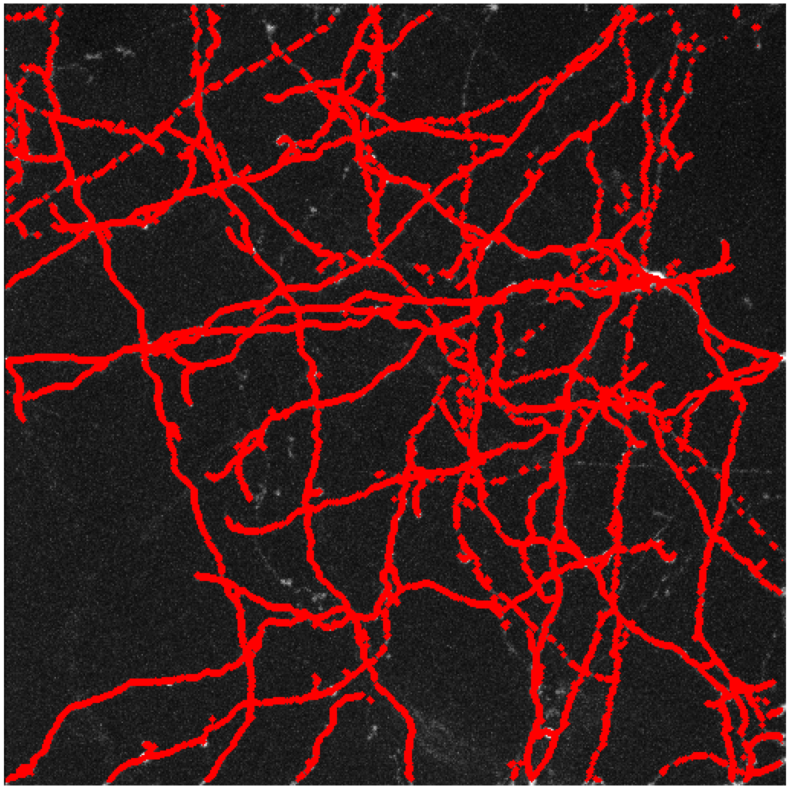
Extracted Neuronal Morphology by optimal MPP object configuration

Cerebellar Climbing Fibres
Transmitted Light Brightfield

Results (S1)



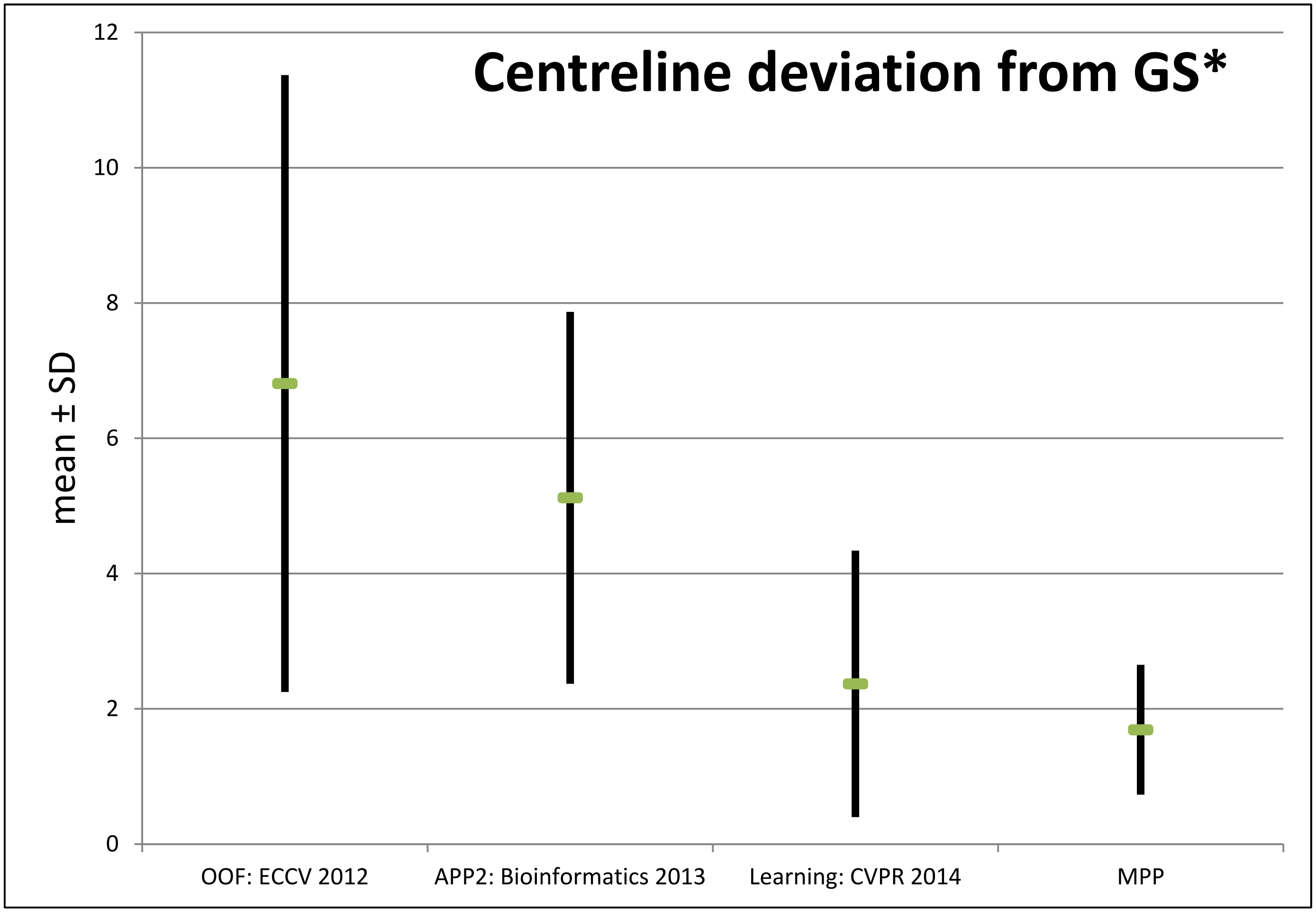
Maximum Intensity Projection



Extracted Neuronal Morphology by optimal MPP object configuration

Neocortical Layer 1 Axons
2-photon lasers scanning microscopy

Evaluation (S1)



*GS: Gold standard

Contribution (S1)

- **Automatic**, **unsupervised** detection; **no user interaction**
- **Connected, single pixel centreline**
- **Special configurations of marked objects** and **energy function**
- Obtains neurite **centreline, local radius and orientation**.
- **Non-maxima suppression** of scale-orientation space of tubularity filters

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- Introduction
- S1. Detection of tubular structure networks
- **S2. Modeling single neurite morphology**
  - **Parameters of the model**
  - **New energy function**
  - **Results**
- S3. Reconstruction of neuronal trees
- Summary

Motivation (S2)

- Parameters of the model
- New energy function

Automatic Parameter Estimation

Chatelain et al EMMCVPR '09

Hadj et al PCV '10

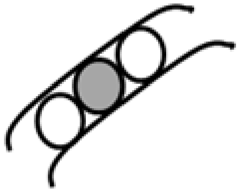
Stochastic Expectation Maximization

Chatelain et al. Statistics & Computing '09

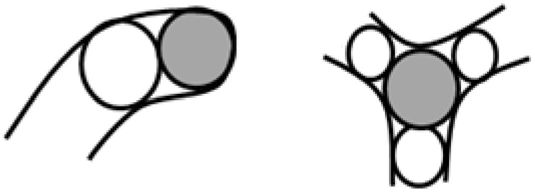
Composite Likelihood Estimator

Least Squares Estimator; Recursive Random Search;
Deterministic Gradient Descent

Improved priors for single neurons



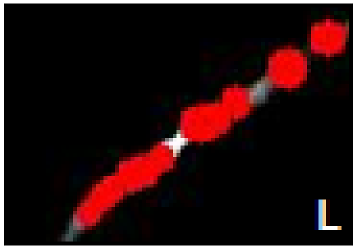
Connectedness

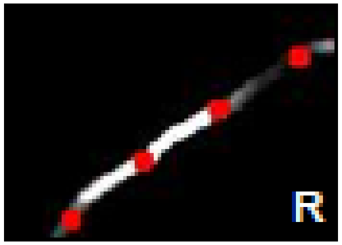


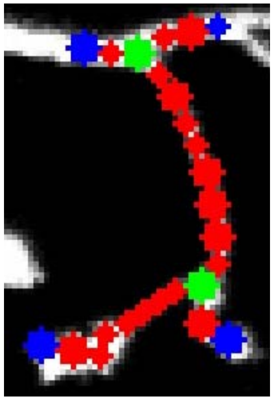
Spatial Configurations

$$U_i(\omega_i, \omega_j) = \begin{cases} U, \text{ if } d < d_r \\ -U, \text{ if } d_r \leq d \leq d_a \\ 0, \text{ if } d > d_a. \end{cases}$$

$$U_c(\omega_i) = \begin{cases} \infty, \text{ if } k(\omega_i) = 0 \\ -E_1, \text{ if } k(\omega_i) = 1,3 \\ \infty, \text{ if } k(\omega_i) > 3 \end{cases}$$







L: Considering radiometry
R: Radiometry + interaction

Basu et al. PRNI 2014

Green : Bifurcation
Blue : Terminal
Red : Intermediate nodes

Parameters : Sensitivity & Robustness

Increasing order of sensitivity

Parameters of the object	**[r_min, r_max]** *OPF~[1,10]* *CCF~[1,25]*	Derived from domain knowledge and imaging resolution information
Parameters of Energy Function	U,E1, dr, da *U=5, E1=2* *dr=r1+r2, da>dr*	Empirically calibrated from small sections of the image
Parameters of Simulation	**δ**, β=1,Δδ=0.999, Δβ=0.998	1. Condition for convergence : Δβ<Δδ<1 2. Independent of temperature 3. δ is a critical parameter

Basu et al.PRNI 2014

Critical : Birth Intensity δ_0

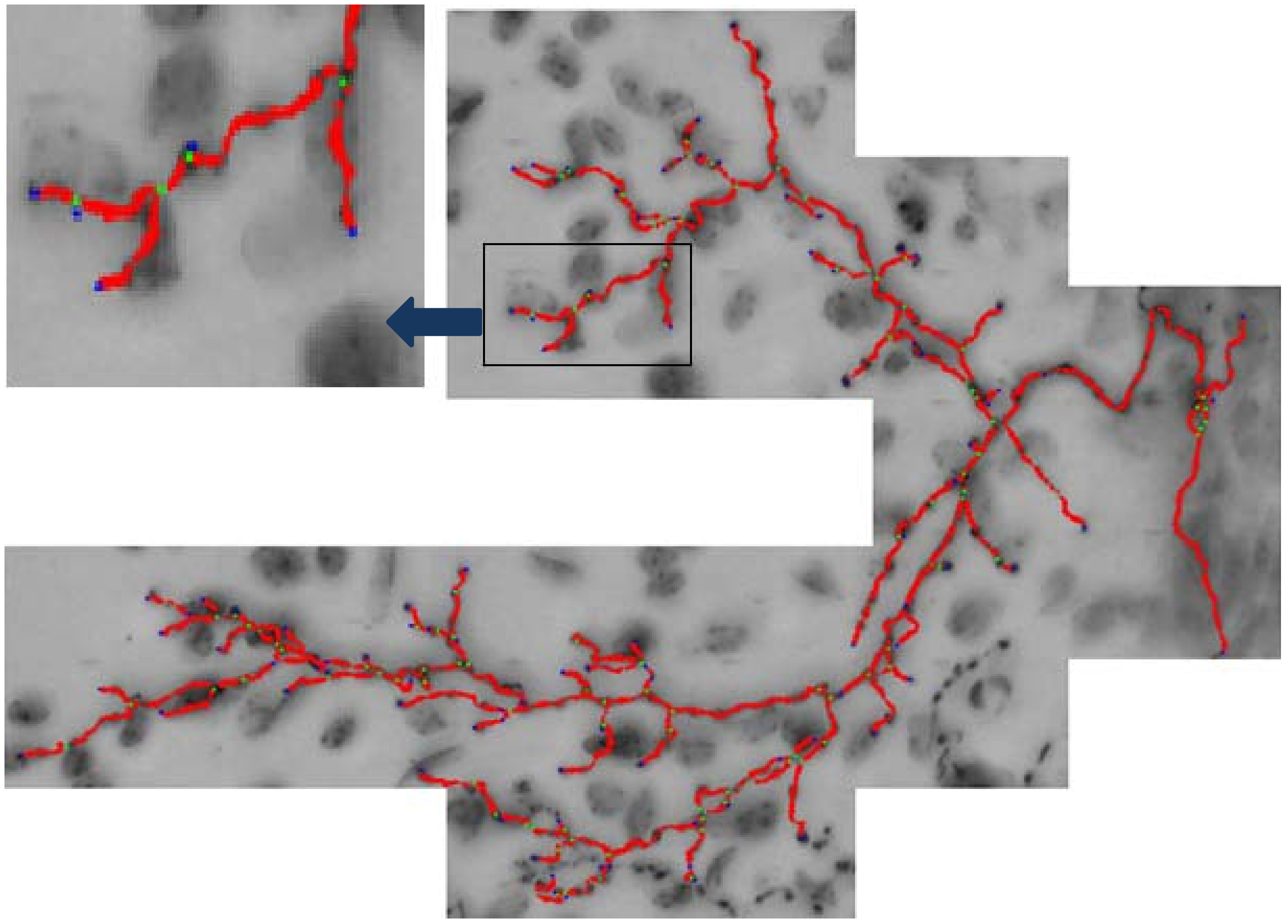
δ_0 : over estimation of #objects in final configuration

1. Clustering – foreground (N_f) and background (N_b)
2. mean radius : $r_m \sim [r_{min}, r_{max}]$
3. Volume of mean sphere $V_m = \frac{4\pi r^3}{3}$
4. Estimated no of objects $N = \frac{N_f}{V_m}$

Results (S2)

Olfactory Projection Fibres : OP5
Extraction of neuronal morphology by MPP objects;
Green->bifurcations, Blue-> terminals

Results (S2)



Cerebellar Climbing Fibres: CCF1
Extraction of neuronal morphology by MPP objects;
Green->bifurcations, Blue-> terminals

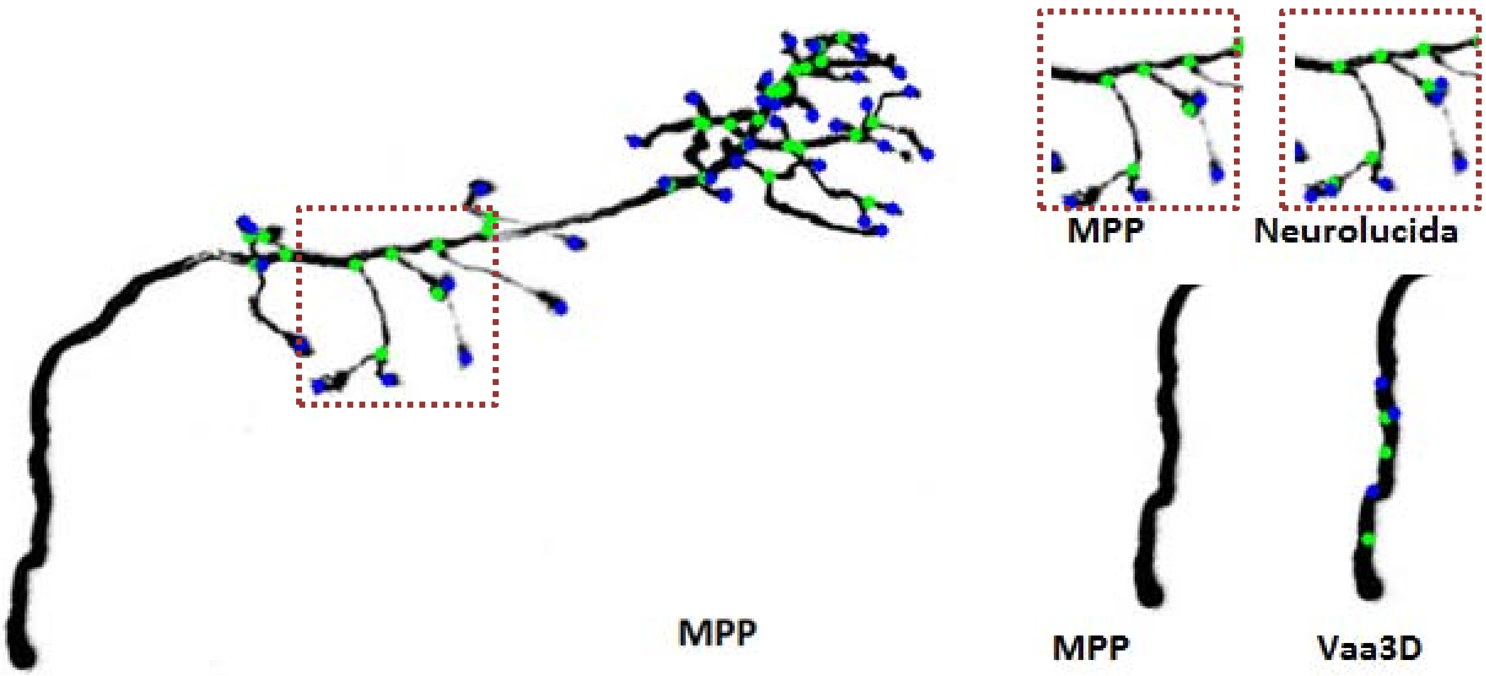
Evaluation (S2)

	SNT (SA)	Neurolucida (SA)	3D tip (A)	Vaa3D (A)	MPP (A)
OP1	41	49	48	43	43
OP4	43	61	46	46	39
OP5	9	9	17	6	9
OP6	18	18	23	12	16

Example of GSR variability : No of terminals reported

A: Automated
SA: Semi-automated

Evaluation (S2)



GSR variability - Vaa3D (A) ; Neurolucida (SA) ; MPP (A)

Contribution (S2)

- Semantic description of neurite morphology
- Identification of critical nodes
- Parametric descriptors for neuronal classes
  - Total length; average branch length
  - Branch tapering rate
  - Branching order; branching angles etc
- Parameter initialization rules
- Parameter dependencies

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- S2. Modeling single neurite morphology
- **S3. Reconstruction of neuronal trees**
  - **Connect MPP nodes as MST**
  - **Verify edges of MST**
  - **Results**
- Summary

Motivation (S3)

- **Reconstruction** objective : Unordered set of points -> Tree
- MPP limited ability in capturing connectivity, MS tree hierarchy

- Image evidence for connecting nodes

Inferring Connected Trees

Active contours
- Roysam et al. Neuroinformatics '11
- Chothani et al. Neuroinformatics '11

Iterative Kernel Fitting
- Zhao et al. Neuroinformatics '11

Skeletonization
Medial Axis
- Lu et. al. PLoS ONE, 2009
- Peng et. al. BMC Bioinformatics 2011

Reconstruction Algorithm

Step 1
- Adjacency matrix with MPP objects as nodes; edge weights as Euclidean distance
- MST (Kruskal's)

Step 2
- Verify edges by recovering it as geodesic minimal path
- If no geodesic minimal path between nodes, remove end node

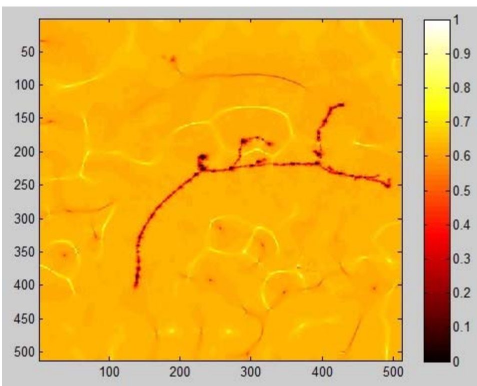
Step 3
- Update Adjacency matrix with reduced node set
- Final MST

Basu et al. ICIP 2014

Verify edges

MST [N,E]

GVF based speed image F(x)

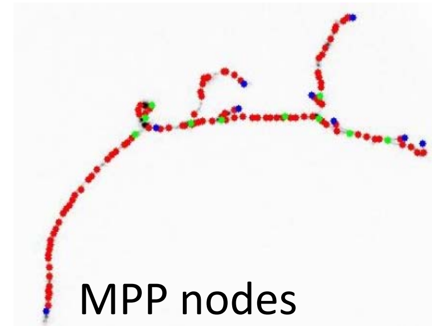


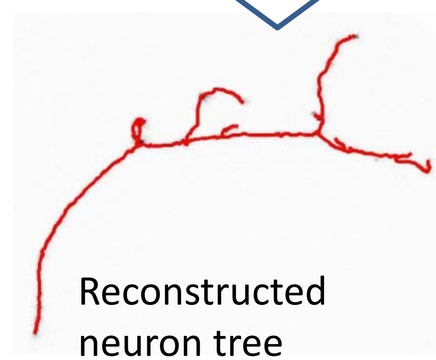
Arrival time map T(x) of front on F(x) from start to end nodes

Gradient descent on T(x) extracting connected minimal path between nodes

remove end node; find new end

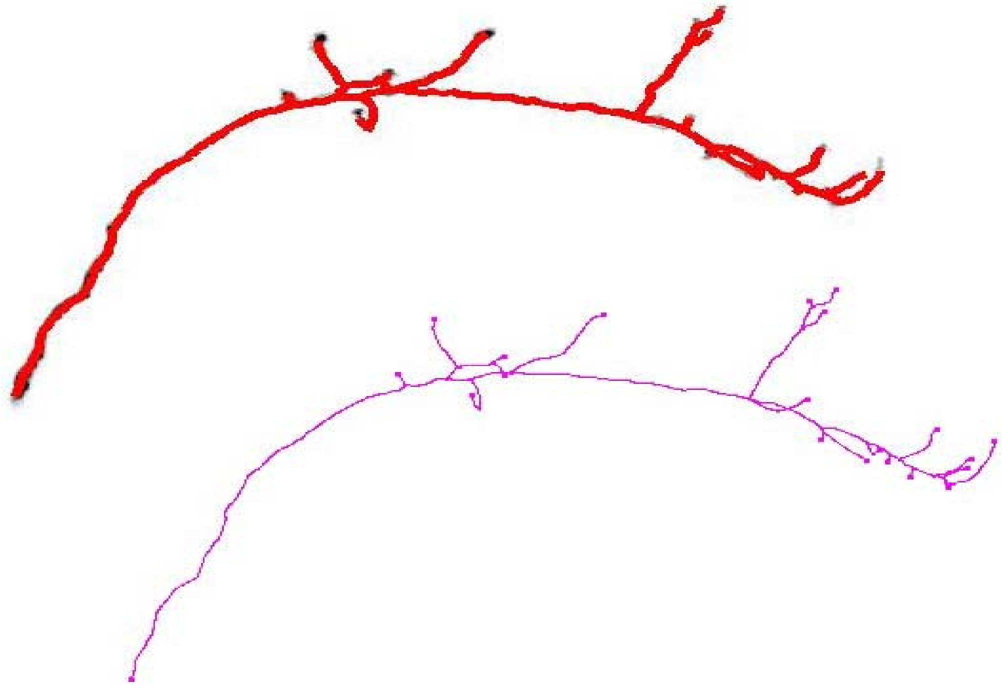
These minimal paths build up a graph representation of the neuronal tree





Basu et al. ICIP 2014

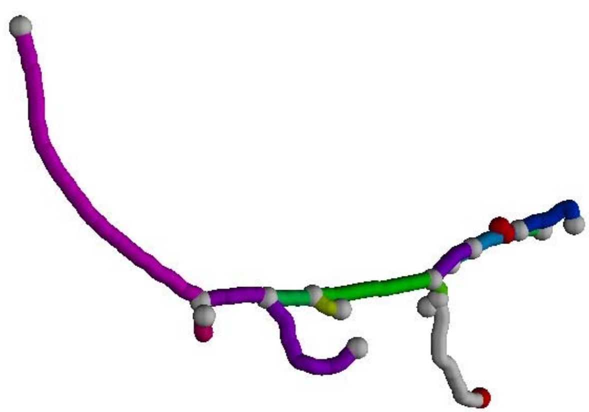
Results (S3)



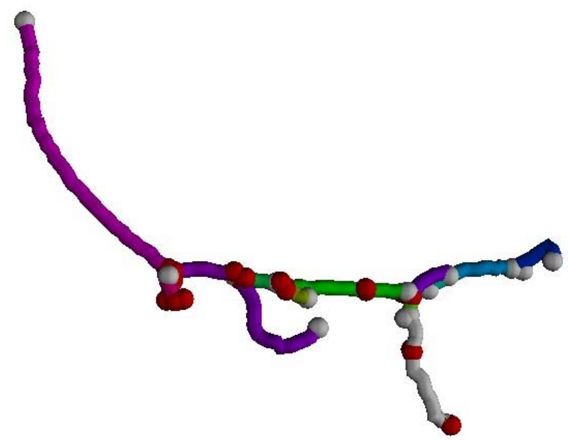
Olfactory Projection Fibres : OP6 by Confocal Microscopy
RED: reconstruction from MPP configuration; PINK:Gold Standard Manual Reconstruction

Evaluation (S3)

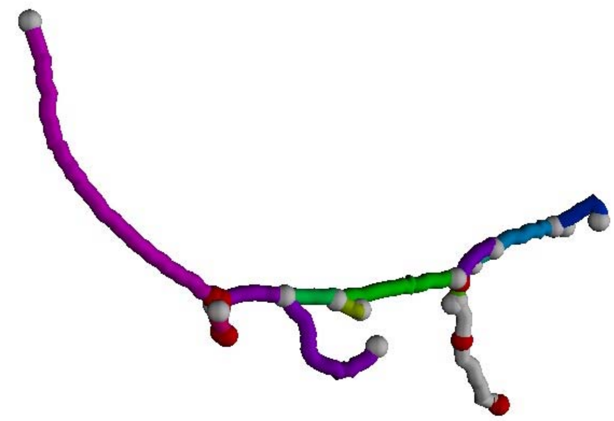
NetMets Visualization



Gold standard manual reconstruction
Red nodes: FN



MPP
Red nodes: FP



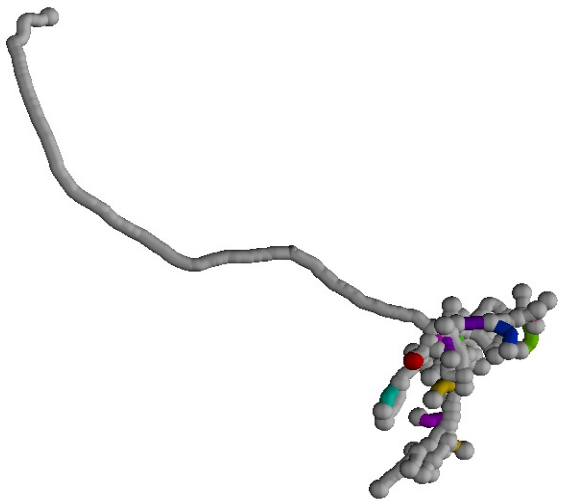
MPP+FFM
Red nodes: FP

Error

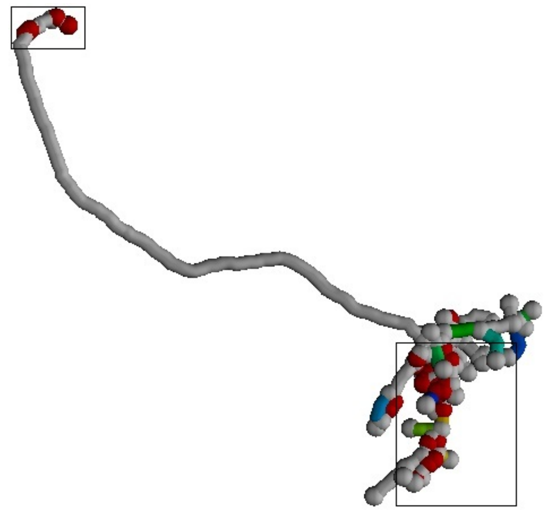
Olfactory Projection Fibres (OP5)

Evaluation (S3)

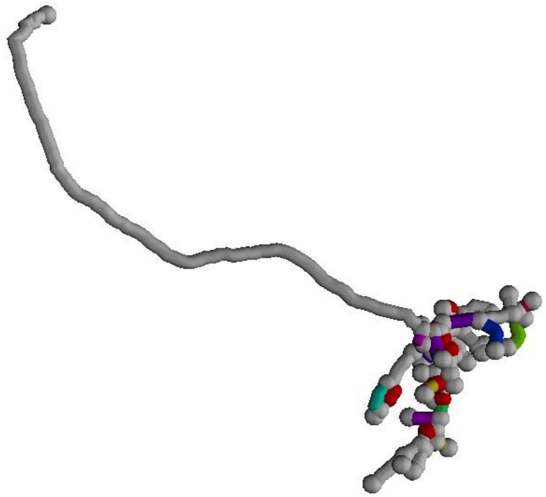
NetMets Visualization



Gold standard manual reconstruction
Red nodes: FN



MPP
Red nodes: FP



MPP+FFM
Red nodes: FP

● Error

Olfactory Projection Fibres (OP5)

Quantitative Evaluation (S3)

NetMets (0-1; lower is better)

g: geometric
c: connectivity
FN: False negative
FP: False positive

	gFN	gFP	cFN	cFP
MPP	0.026±0.005	0.042±0.006	0.35 ±0.17	0.441±0.20
MPP+FFM	0.026±0.005	0.042±0.006	0.327±0.12	0.282±0.12

DIADEM (0-1; higher is better)

F – score
Combining
FP and FN

	OP1	OP4	OP5	OP6	OP7	OP8
MPP	0.829	0.789	0.797	0.830	0.921	0.854
MPP+FFM	0.846	0.807	0.818	0.854	0.926	0.863

Quantitative Evaluation (S3): DIADEM

Method	DIADEM metric score	Extent of automation	Remarks
Average manual tracer	0.78±0.1		
Roysam et. al Neuroinformatics 2011	***0.863±0.35***	SA	
Stepanyants et. al Neuroinformatics 2011	0.80±0.1	SA	
Mukherjee et. al SPIE 2012	0.82±0.07	A	
Xiao et. al Bioinformatics 2013	0.77±0.17	A	
MPP	**0.837±0.043**	**A**	
MPP+FFM	**0.852±0.038**	**A**	
Gala et. al Frontiers of Neurosc. 2014	-	SA, Supervised	No SWC, contains loops

Automatic Semi automatic Manual Best Second best

Contribution (S3)

- Automatic reconstruction pipeline
- Our Marked Point Process (MPP) model detects the branches and Fast Marching connects the MPP nodes into a connected Minimum Spanning Tree (MST).
- Incorporate image evidence in notion of connected nodes
- Remove false positives
- Improves overall accuracy of automated digital reconstruction of neuronal trees

Publications

➢ Detection of tubular structure networks

- Sreetama Basu, Maria S. Kulikova, Elena Zhizhina, Wei Tsang Ooi, Daniel Racoceanu: **A Stochastic Model for Automatic Extraction of 3D Neuronal Morphology.** Medical Image Computing and Computer Assisted Intervention **(MICCAI '13),** Sept 2013

➢ Parameter Initialization and improved priors

- Sreetama Basu, Wei Tsang Ooi, Daniel Racoceanu: **Improved Marked Point Process Priors for Single Neurite Tracing.** International Workshop on Pattern Recognition in NeuroImaging. **(PRNI '14),** June 2014

➢ Reconstruction of neuronal trees

- Sreetama Basu, Daniel Racoceanu: **Reconstructing Neuronal Morphology from Microscopy Stacks using Fast Marching.** IEEE International Conference on Image Processing. **(IEEE ICIP '14),** Oct 2014

➢ [In preparation] Sreetama Basu, Wei Tsang Ooi, Daniel Racoceanu: **Neurite tracing with marked point process.** IEEE Transactions on Medical Imaging

Future Research Directions

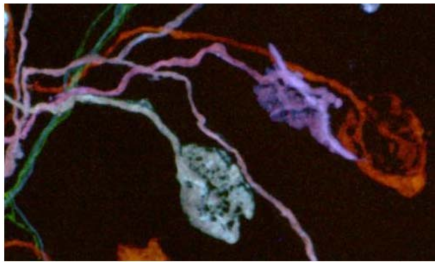
- Massively parallelized processing for high-throughput analysis

Birth (85%) •Eigen Analysis(76%)
Death (7%)

% of execution time in each function

- Parameter space of neuron morphology
  - Quantify inter and intra class difference and similarities

- Multi – neuron interaction



Brainbow ~ Optogenetics

Thesis summary

1. Detection of tubular structure network
   - Automatic, unsupervised MPP framework
   - Global optimum of a well designed energy function

2. Modeling of single neurite morphology
   - Accurate semantic representation
   - Automatic parameter initialization

3. Reconstruction of neuronal trees
   - Connected MST in standard SWC reconstruction
   - Verify edges on image potential

4. Improves overall accuracy of automated analysis and minimizes variation of interactive methods

AUTOMATED ANALYSIS OF NEURONAL MORPHOLOGY: DETECTION, MODELING AND RECONSTRUCTION

Thank you

Thesis summary

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Backup Slides

Energy (Detection)

$$U(\gamma) = \boxed{U_d(\gamma)} + \boxed{U_i(\gamma)} + \boxed{U_c(\gamma)}$$

$$\boxed{U_d(\gamma)} = \sum_{\omega_i \epsilon \gamma} U_d(\omega_i) \qquad U_d(\omega_i) = \left|\frac{\pi}{2} \int_{\theta=0}^{2\pi} \nabla I(x_i + r_i V_\theta) d\theta\right|$$

$$V_\theta = cos(\theta) V_1 + sin(\theta) V_2$$

$$\boxed{U_i(\gamma)} = \sum_{\substack{\omega_i, \omega_j \in \gamma; \\ |\omega_i - \omega_j| < d_a}} U_i(\omega_i, \omega_j) \qquad U_i(\omega_i, \omega_j) = \begin{cases} U_+, & \text{if } d < d_r \\ U_-, & \text{if } d_r \leq d \leq d_a \\ 0, & \text{if } d > d_a. \end{cases}$$

$$\boxed{U_c(\gamma)} = \sum_{\omega_i \in \gamma} U_c(\omega_i) \qquad U_c(\omega_i) = \begin{cases} E_1, & \text{if } k(\omega_i) = 0 \\ -E_1, & \text{if } k(\omega_i) = 1 \\ -E_2, & \text{if } k(\omega_i) = 2, 3, 4 \\ E_1, & \text{if } k(\omega_i) > 4. \end{cases}$$

Multiple Birth and Death Dynamics + Simulated Annealing

Initialization:

Birth intensity **δ0**; Inverse temperature **β0**; Poisson mean **z0**; parameters of energy function; empty start configuration.

Birth:

1. Sample a configuration of spheres $\gamma \in \Omega$ on the image domain and radii independently sampled from $[r_{min}, r_{max}]$
2. Add the new set of objects to ``surviving'' ones to get current configuration .

Death:

1. *Sort objects according to data energy U_d, for accelerating computation;*
2. Objects removed from the configuration is with probability:

$$p(\omega_{i,}\gamma) = \frac{\partial a(\omega_{i,}\gamma)}{1 + \partial a(\omega_{i,}\gamma)}$$

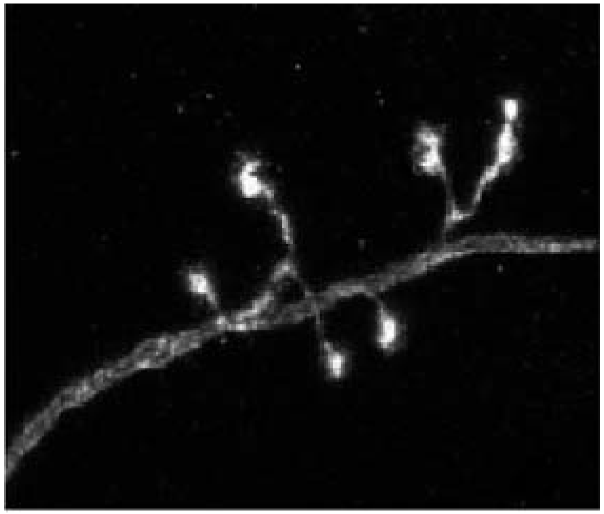
where $a(\omega_{i,}\gamma) = \exp(-\beta(U(\gamma / \omega_i) - U(\gamma)))$

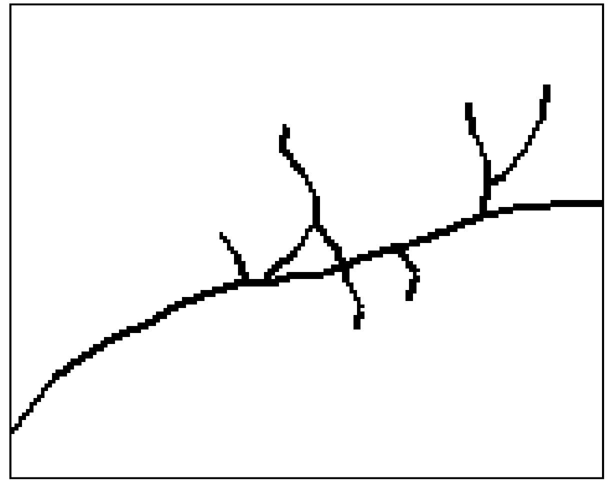
Termination:

If all and only objects added in current iteration are removed in death step, stop. Else, decrease T=1/β,discrete-time step δ; go to birth step.

X. Descombes, R. Minlos, and E. Zhizhina. Object extraction using a stochastic birth-and-death dynamics in continuum. Journal of Mathematical Imaging and Vision, 33(3):347-359, 2009.

State of the art (Detection)



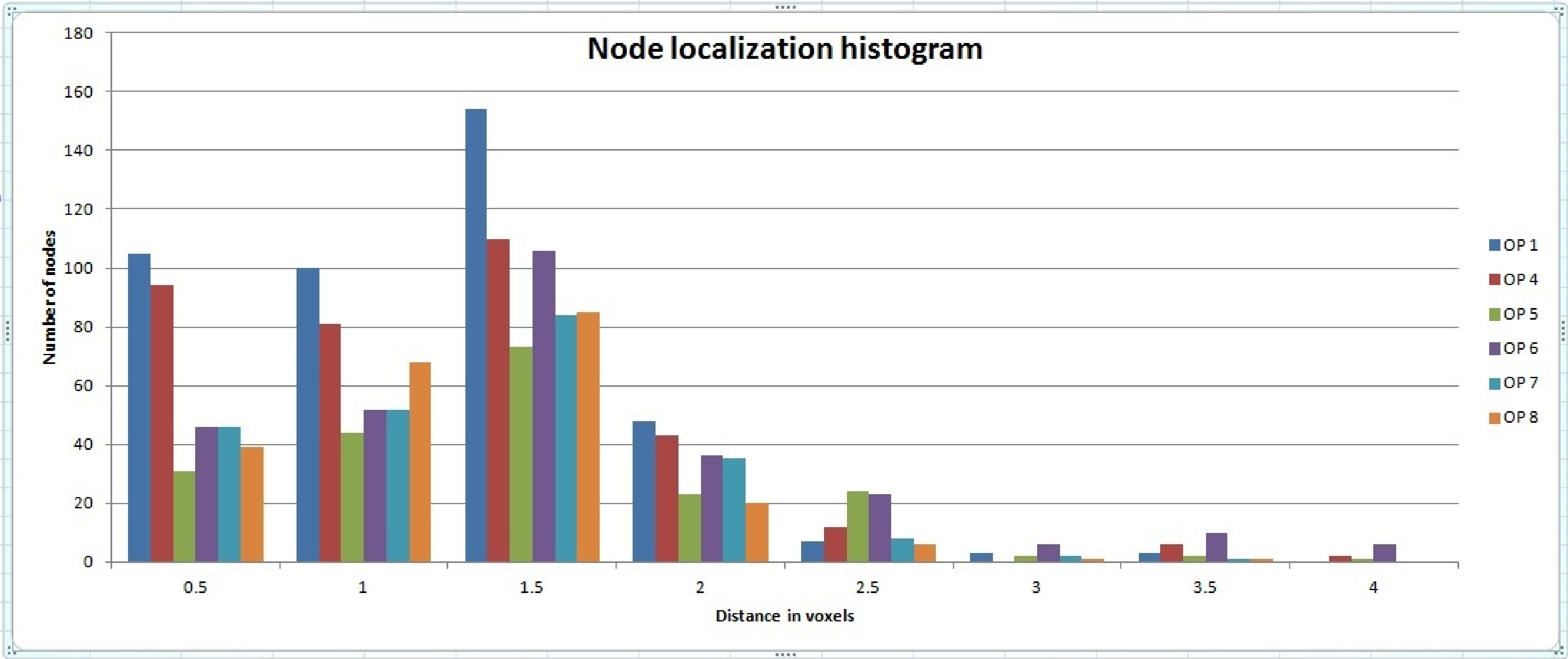


MPP: Basu et.al MICCAI 2013

OOF: ECCV 2012

Classification: CVPR 2014

Evaluation

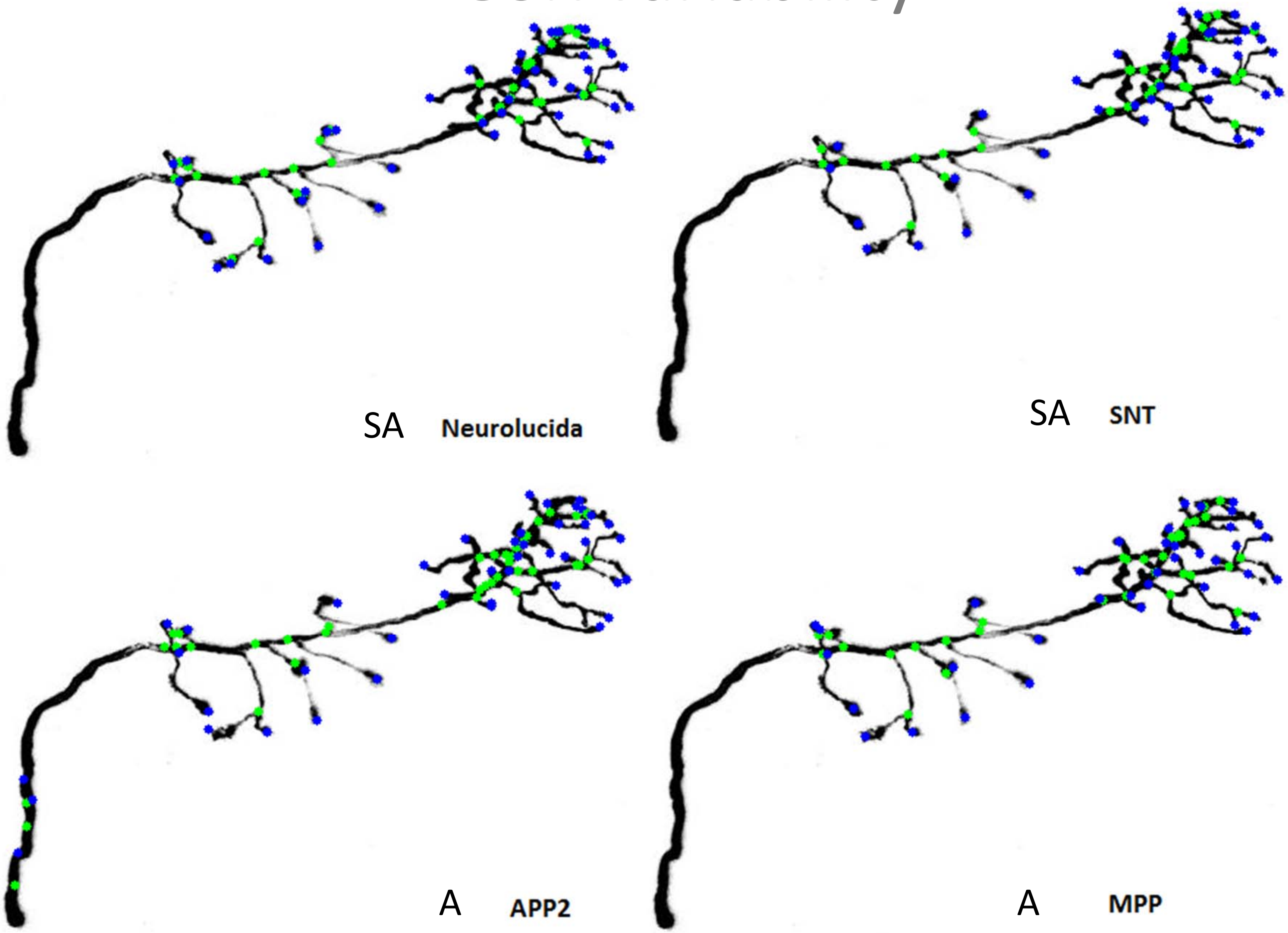


Deviation in euclidean distance between gold standard and our extraction

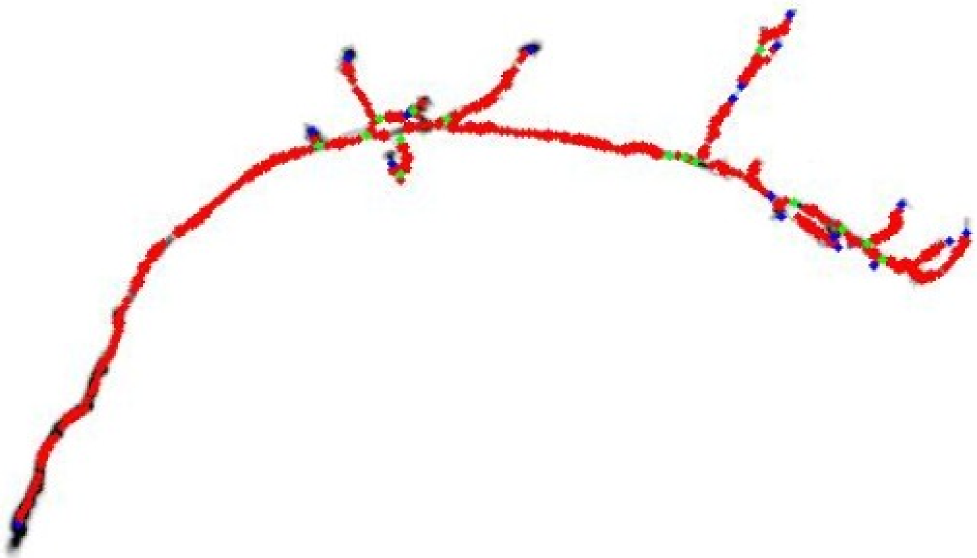
Quantitative Evaluation: Accuracy of nodal identity

	#terminals	FN	FP	#branching	FN	FP
OP1	43/41	5	3	37/39	3	5
OP4	39/43	8	4	40/33	2	9
OP5	9/9	1	1	10/8	1	3
OP6	16/18	2	4	18/17	1	2
OP7	18/16	0	2	15/15	0	0
OP8	10/10	1	1	6/7	1	0

GSR variability



Results (S2)

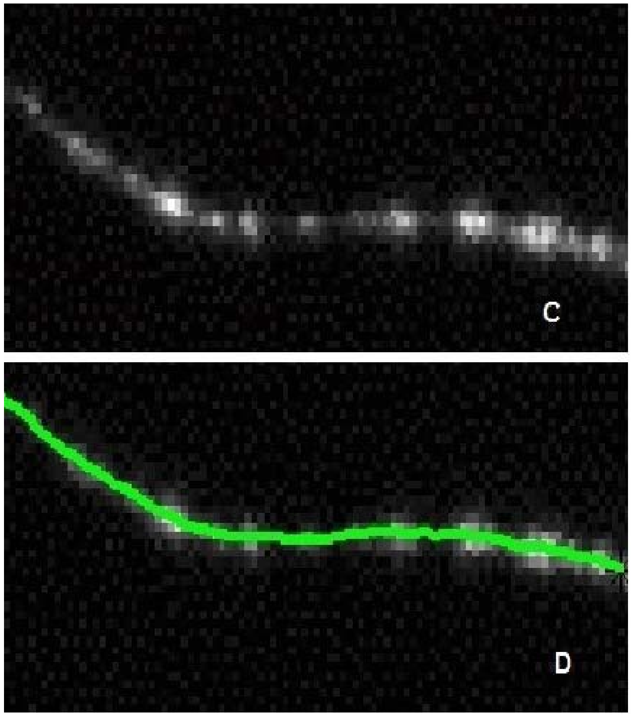


Olfactory Projection Fibres : OP6
Extraction of neuronal morphology by MPP objects;
Green->bifurcations, Blue-> terminals

Fast Marching

- Advantages:
  - Medial axis as branch centerline
  - Inherent connectedness between end points

- Two most critical determinants for front propagation
  - The speed image - F(x)
  - Re-initialization criteria – (MPP nodes)



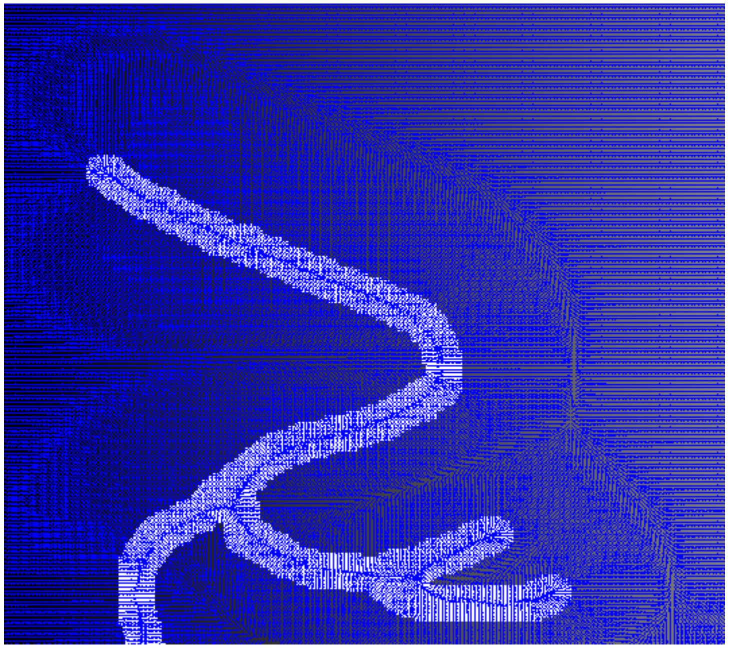
C,D: In spite of beaded appearance of branches (top), a connected minimal path is approximated (in green, bottom).

Gradient Vector Field and speed image

GVF[Xu et. al. MICCAI 1998] : feature preserving diffusion of gradient vector field minimizing the energy -

$$E_{gvf}(x) = \int\int\int_{V^3} (\mu|\nabla V(x)|^2 + |F(x)|^2 |V(x) - F(x)|^2)$$

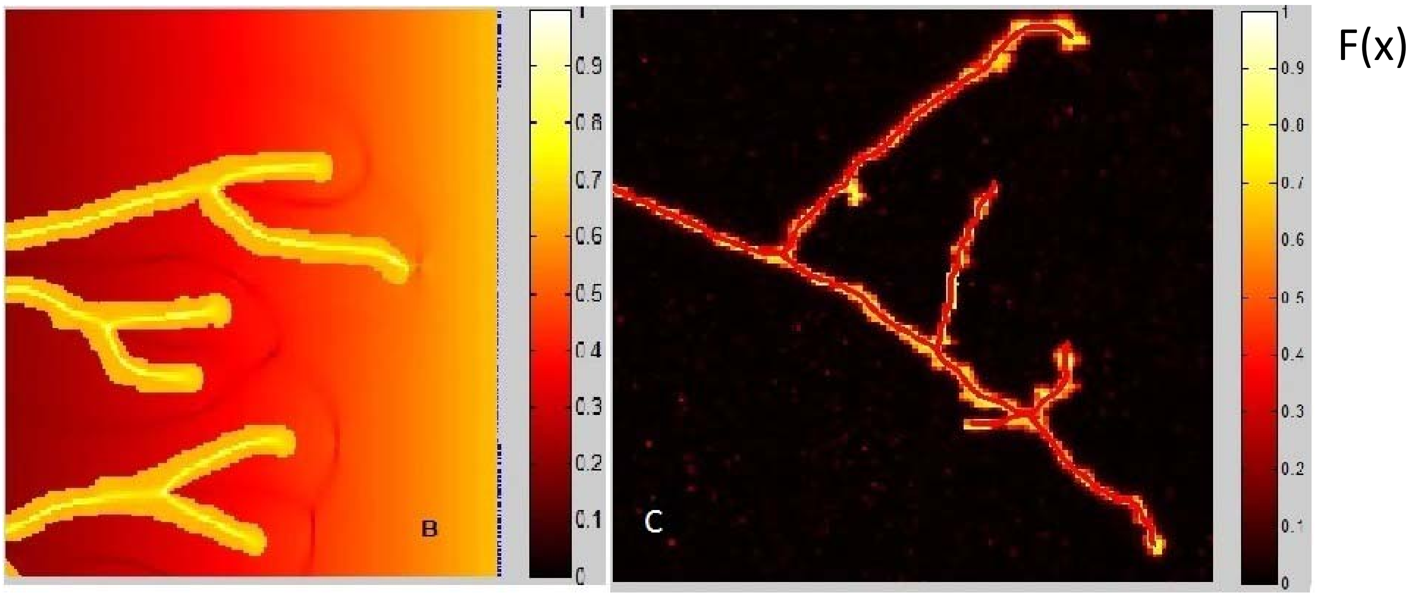
- GVF diffusion accounts for anisotropic voxels
- Vectors convergence at centerline of branches
- No shape assumption on cross-section



Speed Image

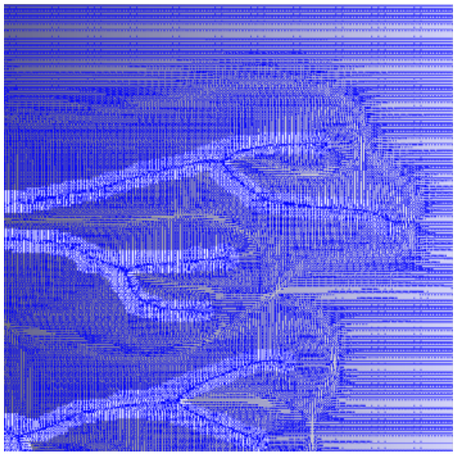
Net Outward Flux : $D(x) = \frac{1}{N_i} \int \int \int_{V^3} V(x_i).\hat{n_i} dS_i$

Speed Image : $F(x) = exp(\gamma[1 - D(x)] * I(x)) - 1$



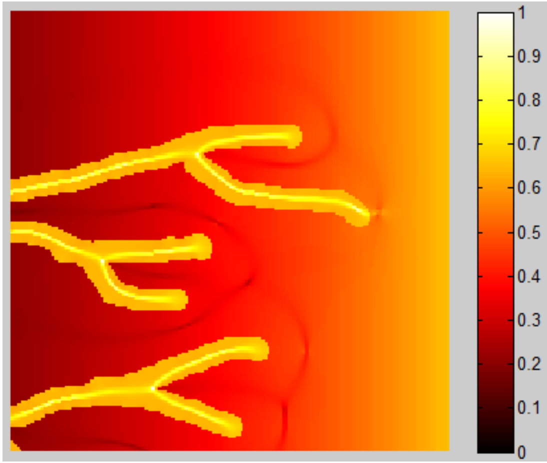
B: The speed image and color map for its interpretation. Propagation speed is highest along the centrelines of the branches. C: Extracted centerlines overlayed in red on speed image.

Centreline as minimal geodesic path



Gradient Vector Field (GVF)

F(x)



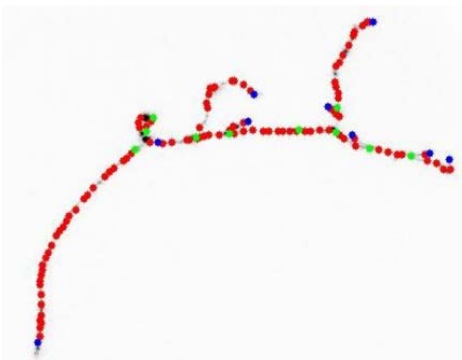
Speed image and color map

Extracted centerlines (red) on speed image.

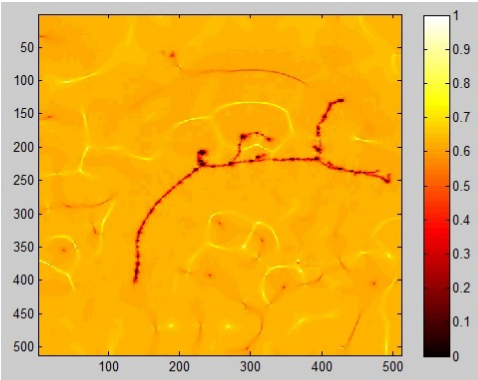
Reconstruction overview

Steps:

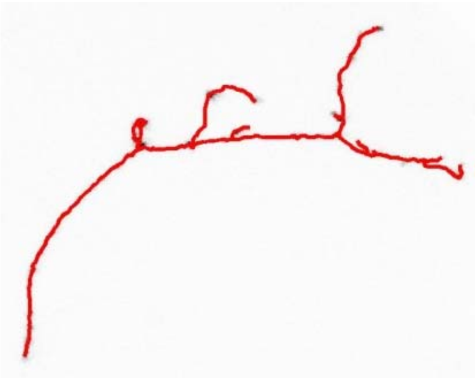
1. Automatically generate seed points by MPP model to re-initialize the marching front.
2. Compute the Gradient Vector Field (GVF)-based speed image F(x).
    a. Generate an arrival time map T(x) with front propagation between sets of nodes on F(x)
    b. Extract the branch topology's medial axis as a geodesic curve by gradient descent on T(x).
3. Reconstruct the neuron tree by connecting the geodesics into a minimum spanning tree.



green : bifurcation,
blue : terminals,
red : anchor.



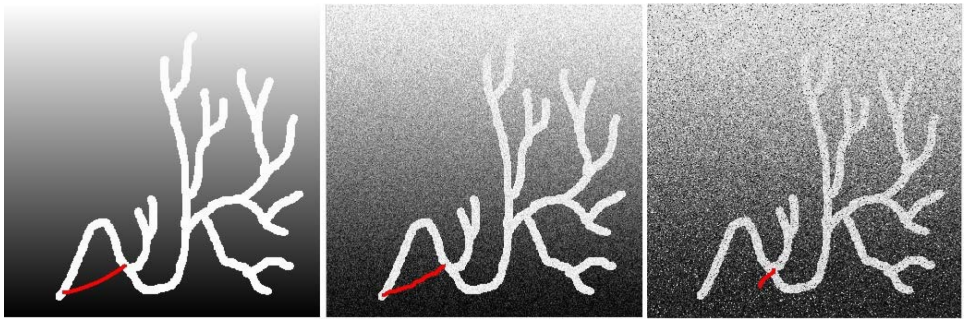
Gradient Vector Field Speed Image

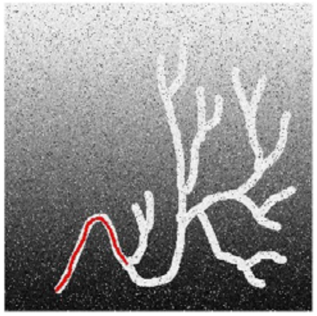


Reconstructed neuron tree

MPP nodes: control points

- Pre-sorted into Minimum Spanning Tree using Kruskal's algorithm.
- Re-initializes propagating front to localize computation and control quality of reconstruction.

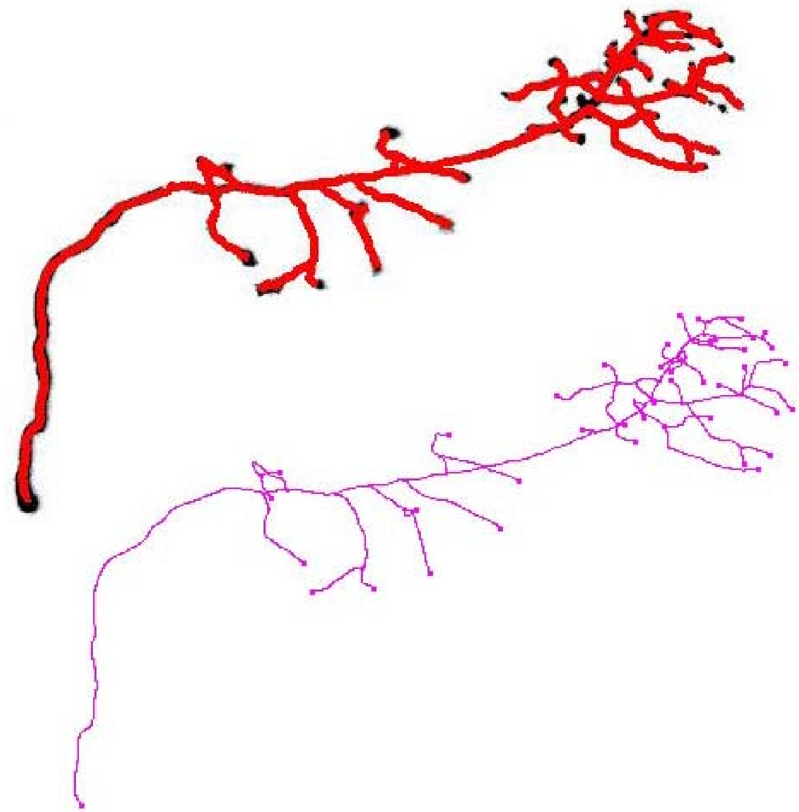




1, 2, 3: with out MPP nodes, GVF speed image; **4**: with MPP nodes as control points on speed image

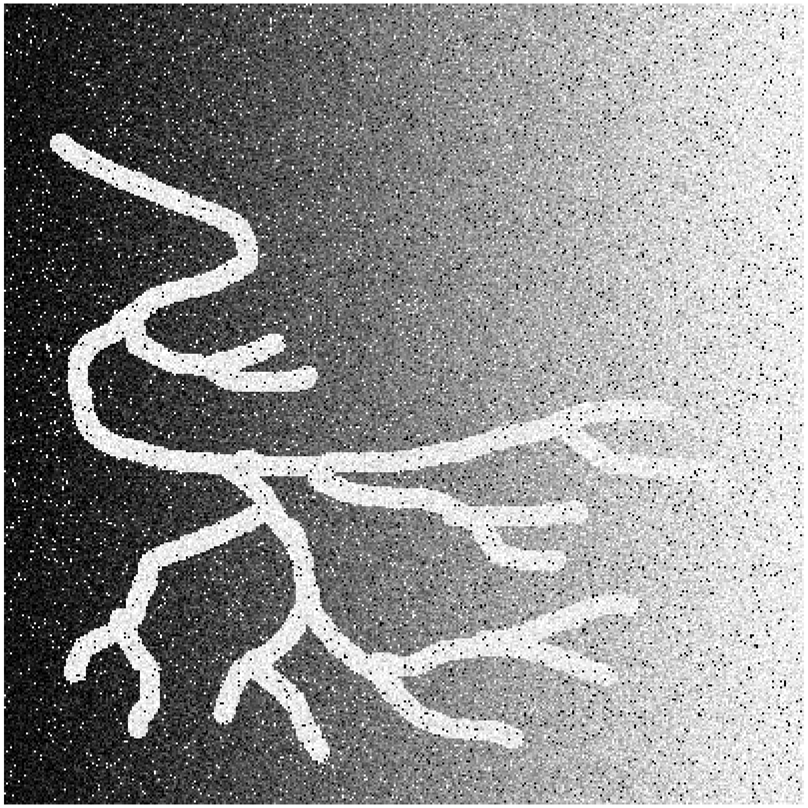
- cellular structures, noise, illumination gradation, cause the front to spill out
- minimal paths cut corners at high curvature branches shortening neuronal length

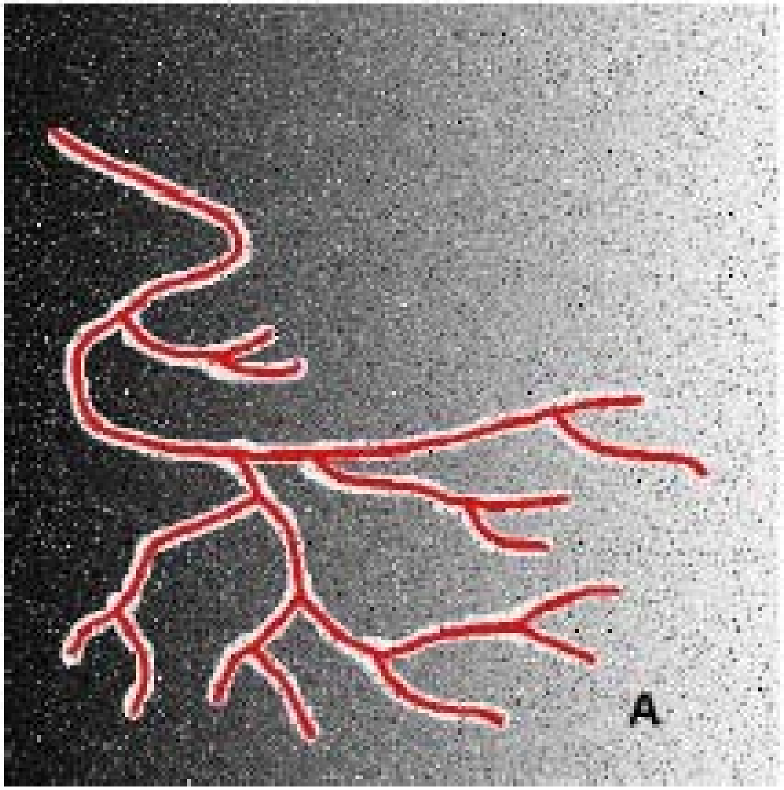
Results (S3)



Olfactory Projection Fibres : OP1 by Confocal Microscopy
RED: reconstruction from MPP configuration; PINK:Gold Standard Manual Reconstruction

Results (S3)





Synthetic 2D data : Synthetic data (SYN01) modeling background gradience due to uneven illumination during image acquisition. The sharp curvature of the branches and added noise make it a challenging task. Reconstructed tree is overlayed in red. B

Quantitative Evaluation: NetMets

g: geometric
c: connectivity
FN: False negative
FP: False positive

MPP

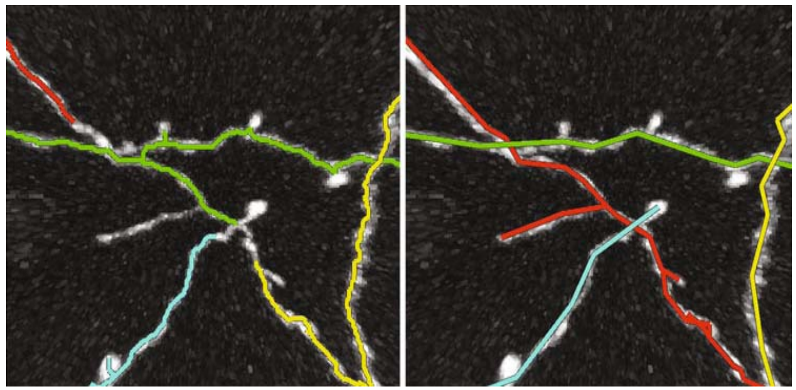
	gFN	gFP	cFN	cFP
OP1	0.028	0.041	0.304	0.313
OP4	0.026	0.052	0.671	0.716
OP5	0.025	0.038	0.176	0.6
OP6	0.052	0.052	0.384	0.416
OP7	0.020	0.037	0.258	0.148
OP8	0.138	0.142	0.328	0.454

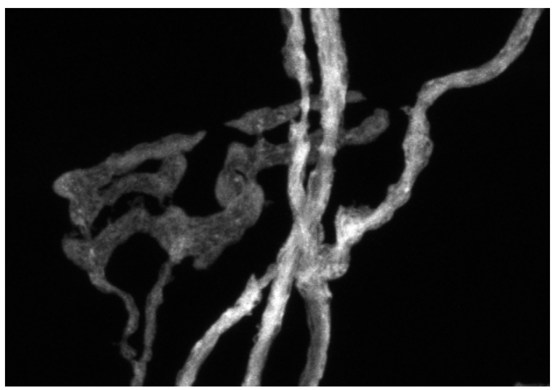
MPP + FFM

	gFN	gFP	cFN	cFP
OP1	0.028	0.041	0.304	0.260
OP4	0.026	0.052	0.546	0.391
OP5	0.025	0.038	0.176	0.333
OP6	0.052	0.052	0.384	0.320
OP7	0.020	0.037	0.225	0.040
OP8	0.138	0.142	0.328	0.352

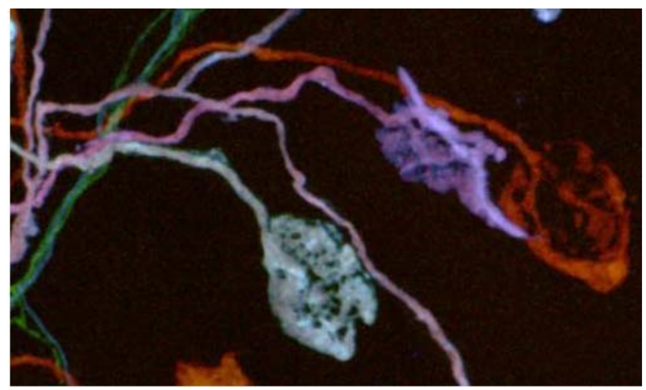
Multiple Overlapping Neurons

Tree stealing (with multiple dendritic trees all similarly labeled)





Cytoplasmic YFP



Brainbow ~ Optogenetics

Neuromuscular Junctions

Errors

Where our algorithm performs poorly?

- Branches almost perpendicular to Z-axis
- High density of labeled neurons
- Very acute branching angles
- Small tertiary branches