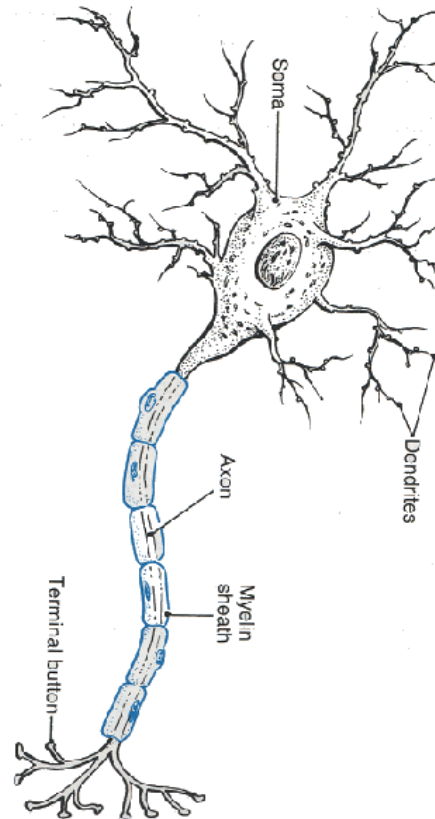


Neuron tracing



Overview

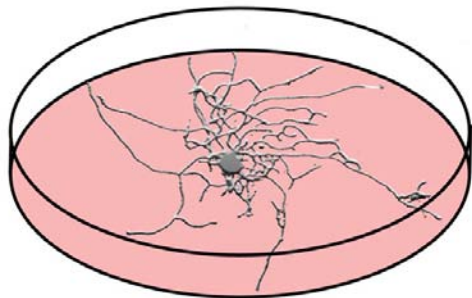
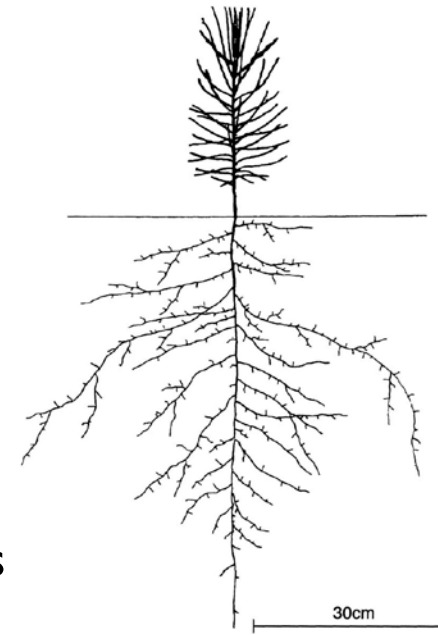
- Introduction
- Applications
- Analysed parameters
- Tracing Software
- Requirements
- Problems

Introduction

- Tracing:
 - to detect, visualize and measure branched structures in 2D and 3D images.
- Applications:
 - Neurite tracing: detecting and measuring dendritic or axonic outgrowths of a neuron in cell culture or in living or fixed tissue.
 - Tracing blood vessels, roots etc.
- Questions:
 - Cell morphology: cell body size, number of neurites, number of branching points, total length, thickness, area covered
 - Connectivity

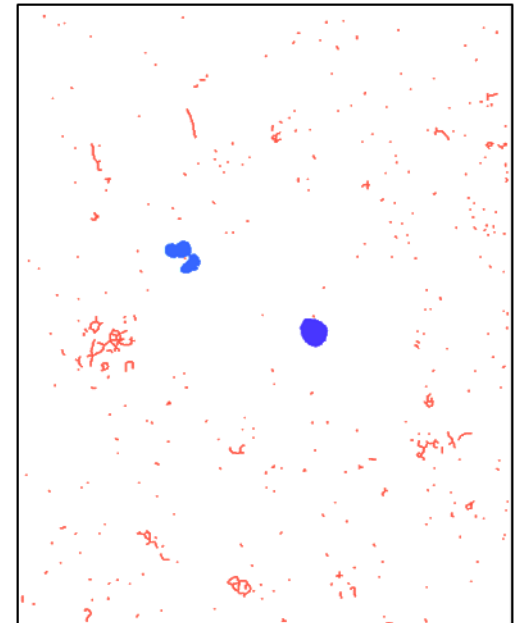
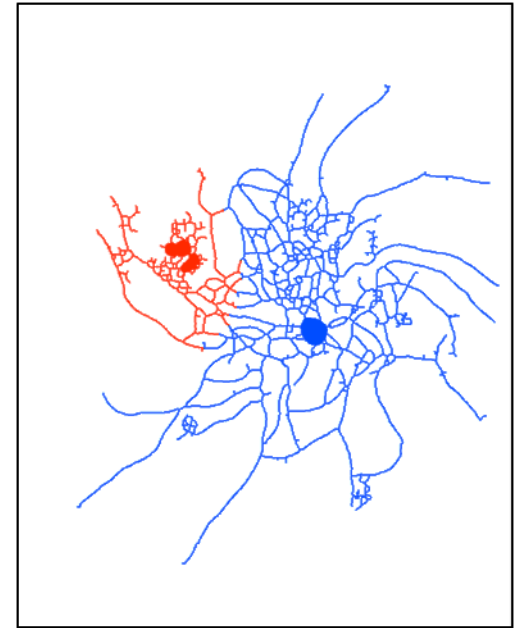
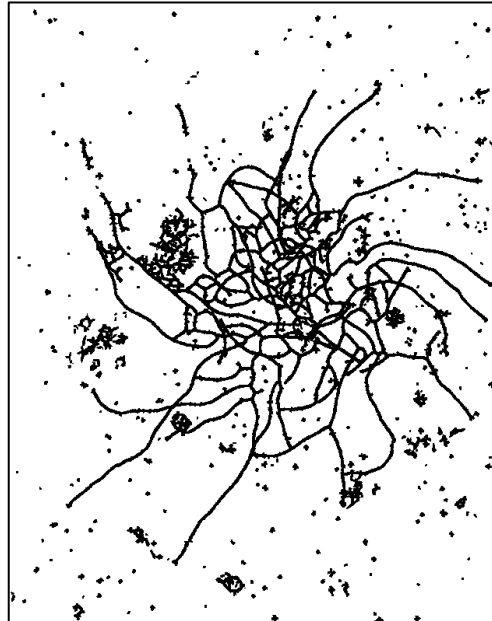
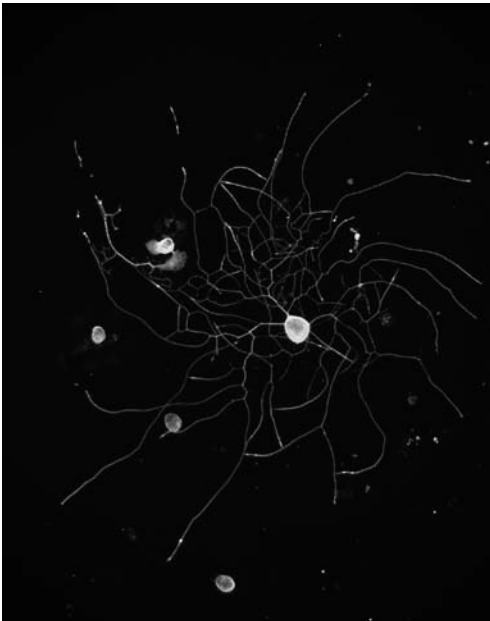
Applications

- Tissue culture of neuron cells as a model
 - 2D tracing
- Tracing of neuronal projections in whole brains
 - 3D tracing
 - Physical or optical sectioning
- Blood vessels, roots or other branched structures



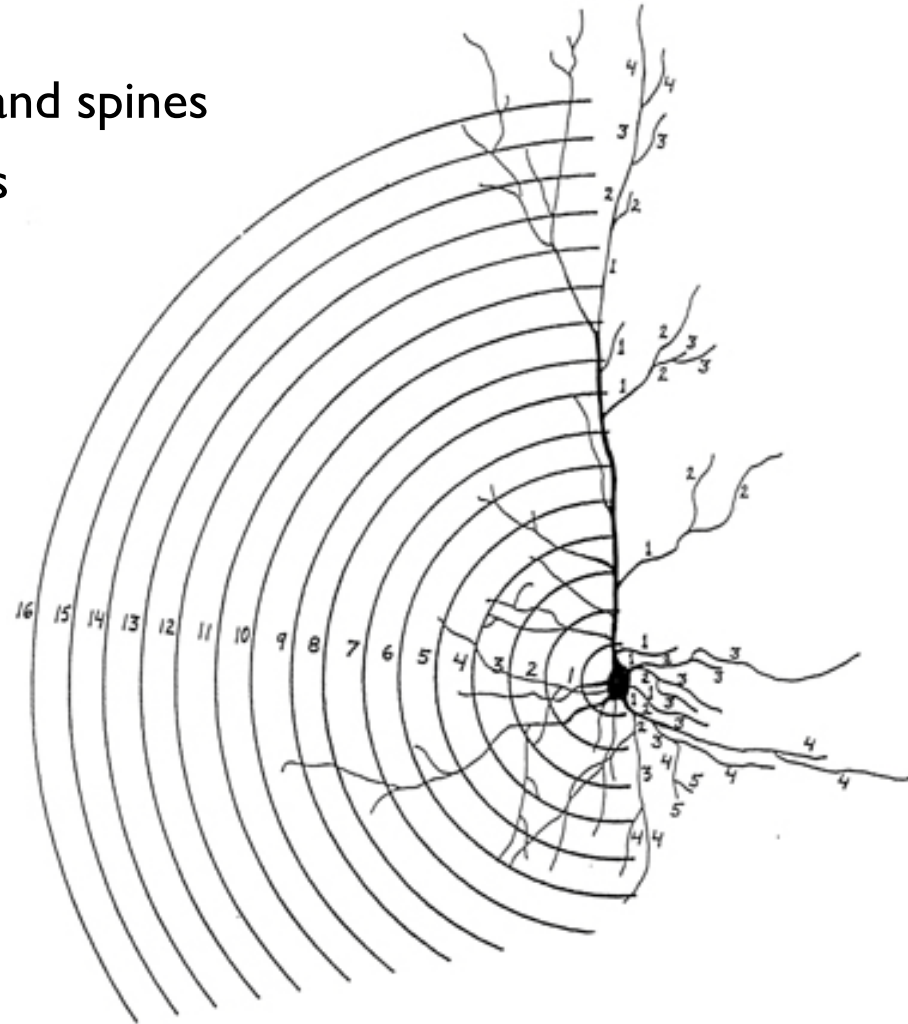
Automatic tracing algorithms

- Edge detection filter
- Detection of parallel lines
- Thresholding image (binarizing)
- Detection of cell body
- Thinning of branches



Analysed parameters

- Total branch length
- Number of cells, dendrites and spines
- Number of branching points
- Branching orders
- Orientation (angles)
- Shape of spines
- Sholl Analysis

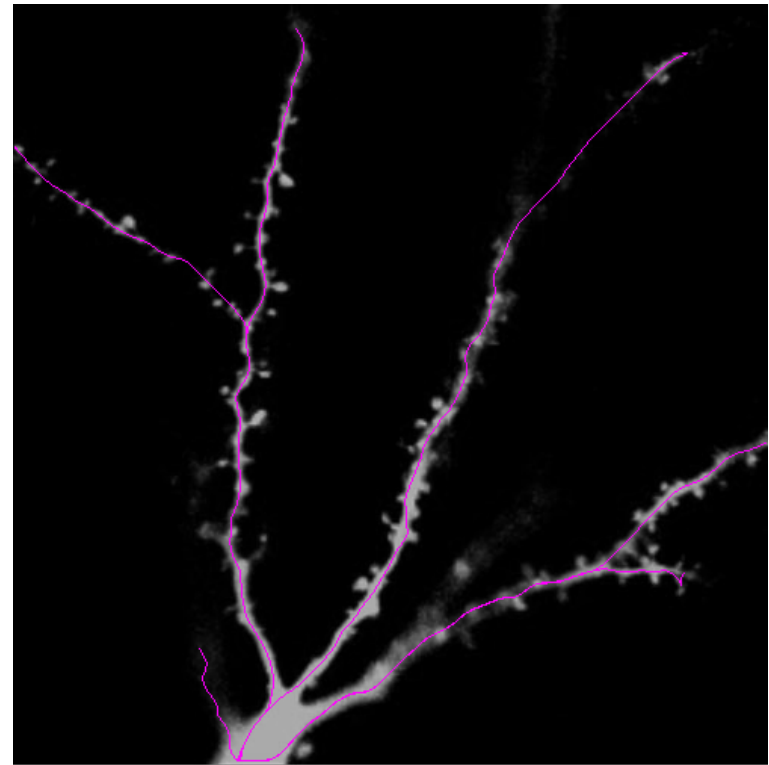


Tracing Software

- ImageJ Plugins
 - NeuronJ
 - Neurite Tracer
 - Simple Neurite Tracer
- Wis-Neuromath
- Imaris
- Amira

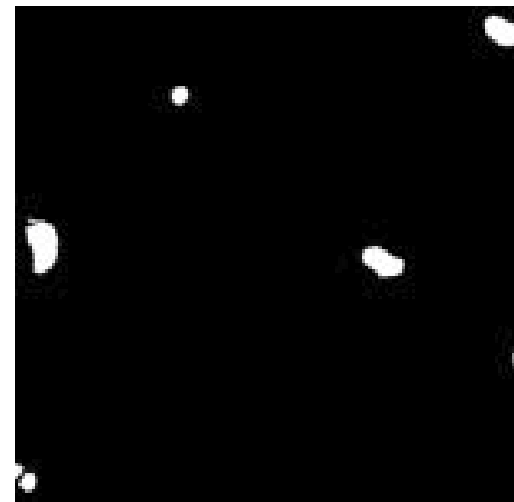
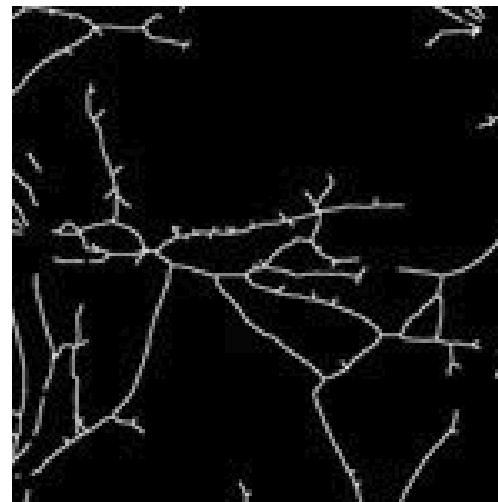
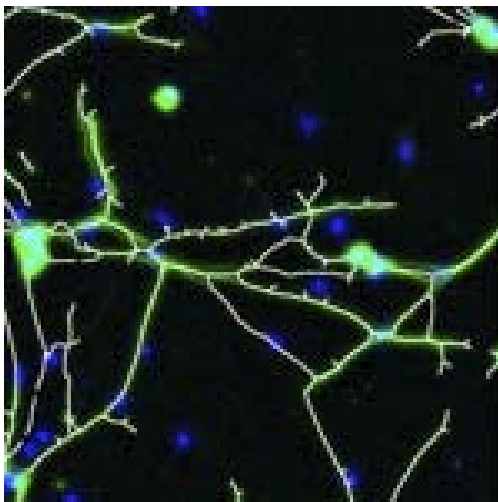
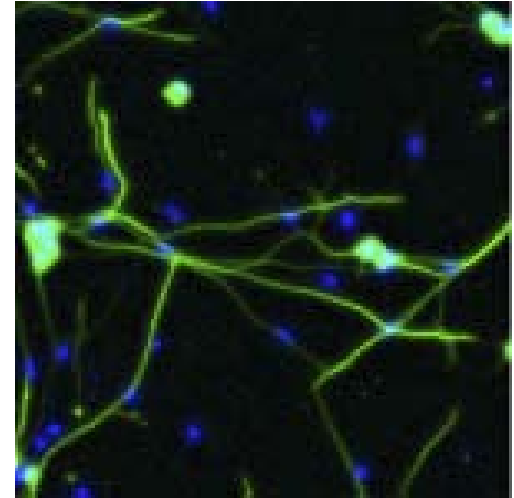
Plugin NeuronJ

- Freeware
- Manual Neuron tracing
- 3D: No
- Batch processing: No
- Only suitable for a low sample number
- Not too complex structures
- Output: length of dendrites



Neurite Tracer

- Freeware
- Automatic image processing
- 3D: No
- Batch processing: Yes
- Output: Number of cells, length of dendrites
- Nuclei staining needed



Simple Neurite Tracer

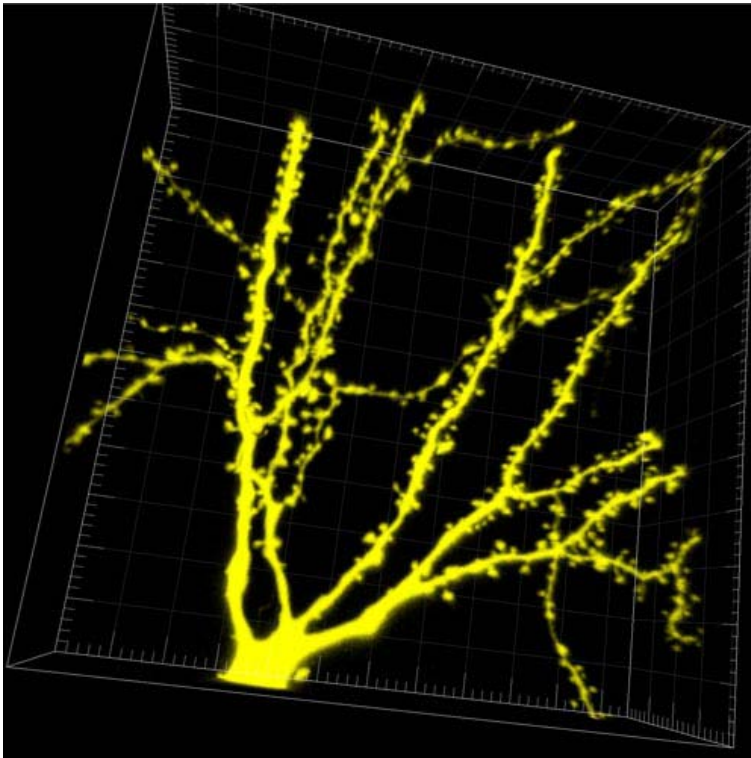
- Freeware
- Manual image processing
- 3D:Yes
- Batch processing: No
- Output: Number of Nuclei, length of dendrites

Wis Neuromath

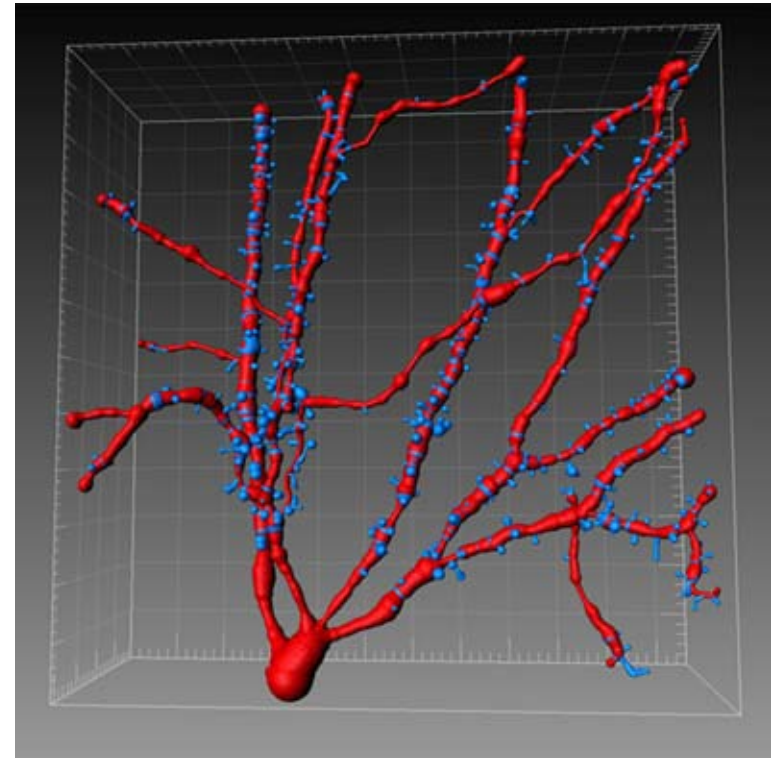
- Freeware
- Automated Image Processing
- 3D: No
- Batch processing: Yes
- 3 main functions:
 - Cell morphology
 - Neurite Length Analysis (in tissue)
 - Ganglion explant Analysis (similar to sholl analysis)

Imaris

- Filament tracer tool embedded in Software



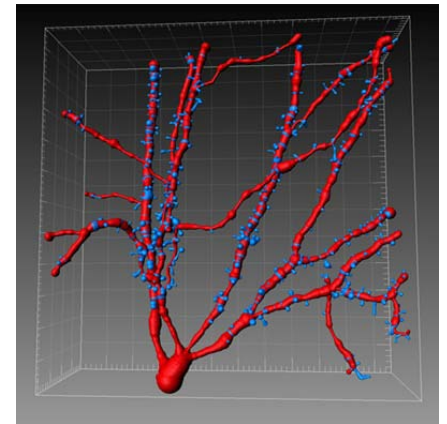
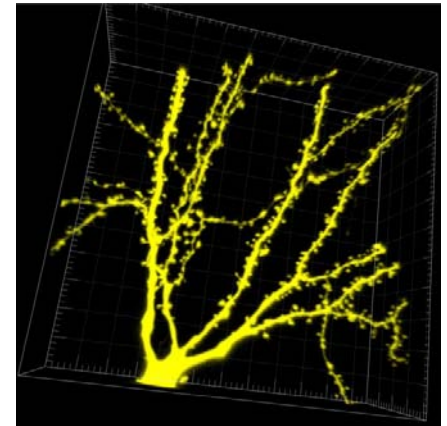
3D-MIP rendering



Dendrite and Spine reconstruction

Imaris

- Freeware: No
- Automated and manual processing
- 3D reconstruction of dendrites and spines
- Batch processing: No
- Output:
 - Detailed Spine analysis
 - Dendrite length
 - Volumes
 - Thickness of dendrites



3D-MIP rendering

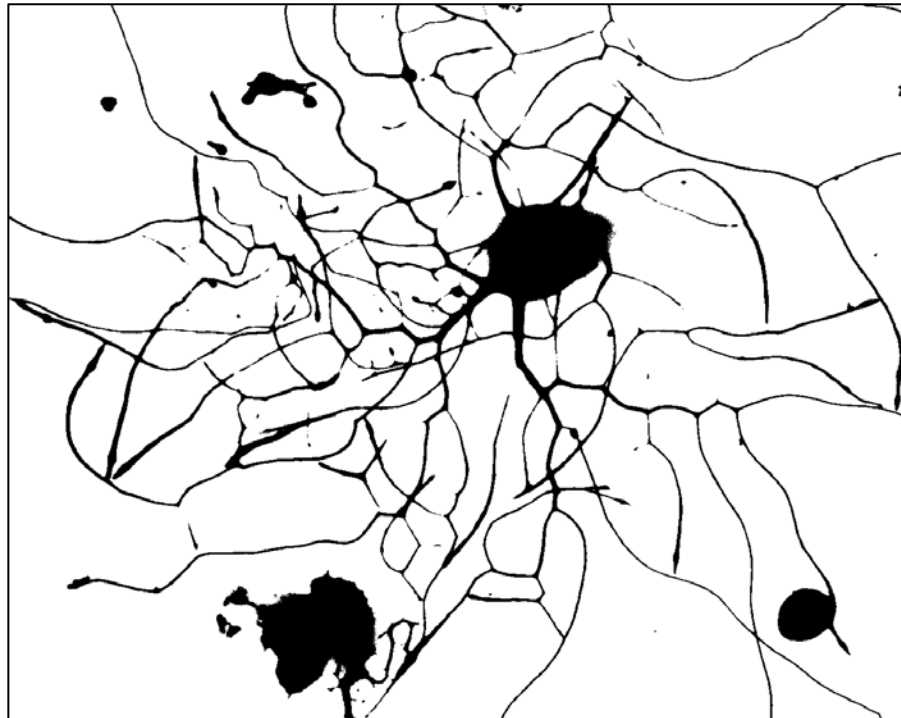
Dendrite and Spine reconstruction

Amira

- Freeware: No
- Automated and manual processing
- 3D reconstruction of dendrites and spines
- Batch processing: No
- Output:
 - Dendrite length
 - Volumes?
 - Thickness of dendrites?

Requirements

- Good contrast (improvement by filtering)
- Well aligned slices in 3D for physical sections (Image Registration)
- Binarizing is necessary for some programs

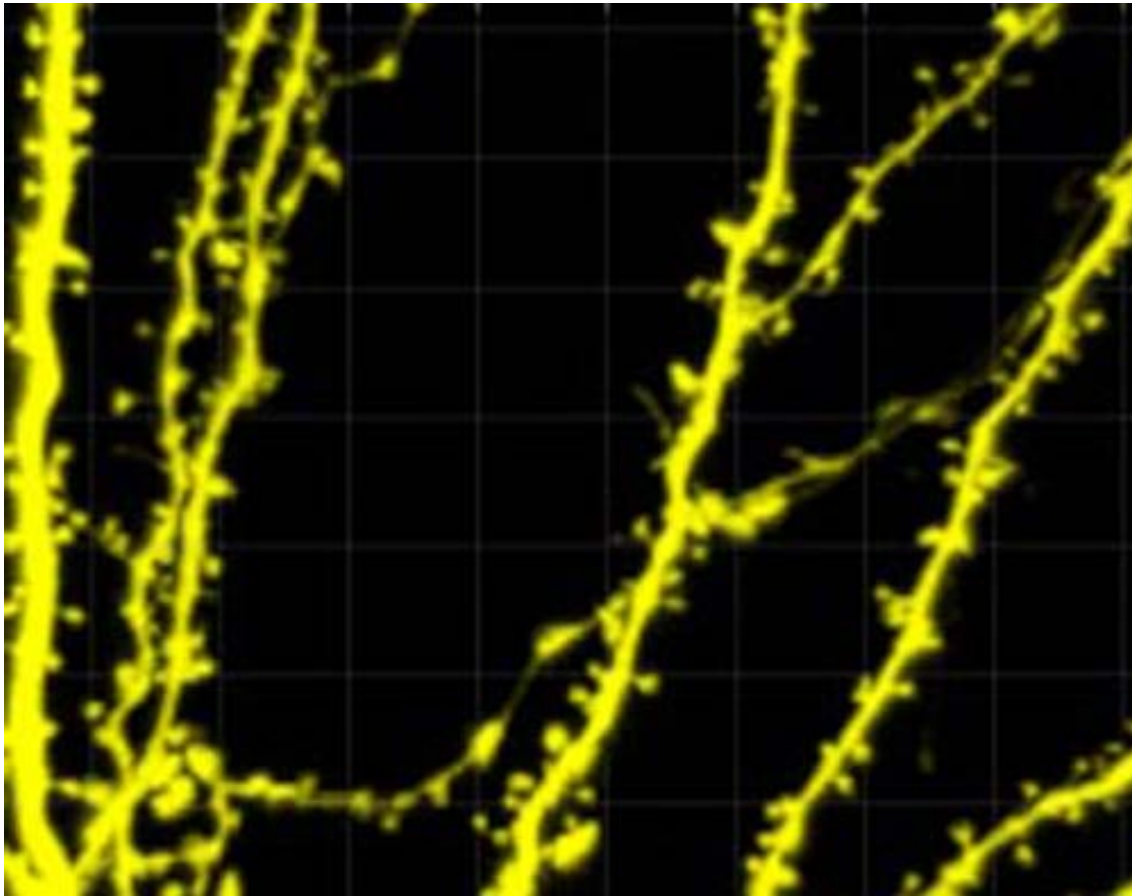


Pitfalls

- Imaging problems
 - Depth of whole tissue – loss of brightness in z
 - Resolution of small structures
 - Low signal to noise ratio
 - Problem of alignment in physical sections
 - Uneven distribution of fluorescence
- Segmentation problems
 - Overlapping of dendrites
 - Weak or thin connections (see imaging problems)

Pitfalls

- Low local contrast leads to disconnected dendrites and spines



Sources

- www.wikipedia.org
- <http://fiji.sc>
- Madeline Pool et al.: NeuriteTracer: A novel ImageJ plugin for automated quantification of neurite outgrowth