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# Three-Dimensional Neuron Reconstruction from Confocal Z-Stacks (Neurolucida)

In 1 collection

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol describes three-dimensional reconstruction of single juxtacellularly labeled neurons from confocal z-stack images using Neurolucida. Reconstructions include the somatodendritic domain and axonal arbors, including local collaterals. Serial sections are aligned for continuity, and shrinkage correction along the z-axis is applied as needed to account for histological processing.

## Guidelines

4. **Trace Axons and Local Collaterals****
- Trace axons from their origin at the soma or proximal dendrites.
- Include local collaterals within the VTA when present.
- Follow long-range axons until they enter major fiber pathways or fluorescence signal becomes insufficient for reliable tracing.

5. **Align Serial Sections****
- Use Serial Section Manager to align fragments from each section into a unified 3D reconstruction.
- Verify continuity of processes across section boundaries.

6. **Apply Shrinkage Correction****
- Apply correction factors for tissue shrinkage along the z-axis as appropriate for the histological workflow.
- Use a consistent correction method across all reconstructed neurons.

7. **Extract Morphometric Parameters****
- Use Neurolucida Explorer to compute morphometric features such as dendritic length, branch points, soma position, and axon length within the traced domain.

## Materials

- Confocal microscope capable of z-stack acquisition.
- Image files (z-stacks) covering the neuron across serial sections.
- Neurolucida (MBF Bioscience).
- Serial Section Manager (Neurolucida module).
- Neurolucida Explorer for morphometric analysis.

## Procedure

1 Acquire confocal z-stacks across all sections containing the labeled neuron.
Use consistent imaging settings within the dataset.
Ensure sufficient z-step resolution to reliably follow dendrites and axons.

2 Import z-stacks for each section into Neurolucida.
Define spatial scale parameters using microscope metadata.

3 Trace soma and dendrites in 3D from z-stacks.
Continue tracing until dendrites reach natural tapering endpoints.

4 Trace axons from their origin at the soma or proximal dendrites.
Include local collaterals within the VTA when present.
Follow long-range axons until they enter major fiber pathways or fluorescence signal becomes insufficient for reliable tracing.

5 Use Serial Section Manager to align fragments from each section into a unified 3D reconstruction.
Verify continuity of processes across section boundaries.

6 Apply correction factors for tissue shrinkage along the z-axis as appropriate for the histological workflow.
Use a consistent correction method across all reconstructed neurons.

7 Use Neurolucida Explorer to compute morphometric features such as dendritic length, branch points, soma position, and axon length within the traced domain.

## Outputs

8 3D neuron reconstruction files in Neurolucida format.
Traced soma, dendrites, and axons including local collaterals.
Morphometric parameter exports from Neurolucida Explorer.

## Critical Steps and Notes

9 Exclude neurons with incomplete fills.
Maintain consistent tracing criteria across neurons.
Visually confirm accurate alignment of serial sections.
Apply shrinkage correction consistently when comparing morphometrics.