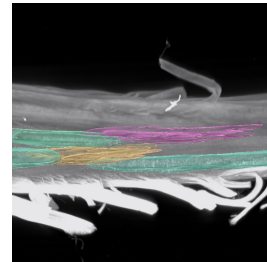


Jan 08, 2026

Segmentation of motor pools in cleared spinal cord

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvmenkb3p/v1>



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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 99160

Keywords: spinal cord, imaris, motor neurons, motor pools, somatic motor neurons, motor columns, autonomic, rat, light sheet microscopy, 3d segmentation of motoneuron pool, spinal cord cytoarchitecture, intact spinal cord of the rat, segmentation of motor pool, motoneuron pool, cleared intact spinal cord, cholinergic neuron identification, cleared spinal cord, 3d segmentation, segmentation, microscopy, reference materials for motor pool identification, motor pool identification, motor pool, light sheet microscopy

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Abstract

This protocol is for the 3D segmentation of motoneuron pools in large-volume images (light sheet microscopy) of the cleared intact spinal cord of the rat. Segmentation is based on cholinergic neuron identification and spinal cord cytoarchitecture, relying on reference materials for motor pool identification.

Citation

Henrik Daa Schrøder (1980). Organization of the motoneurons innervating the pelvic muscles of the male rat. *Journal of Comparative Neurology*.

<https://doi.org/10.1002/cne.901920313>

LINK

Citation

Charles Watson, George Paxinos, Gulgun Kayalioglu, Claire Heise (2009). Chapter 15 - Atlas of the Rat Spinal Cord. *The Spinal Cord*.

<https://doi.org/10.1016/B978-0-12-374247-6.50019-5>

LINK

Materials

Software	
Imaris	NAME
Bitplane	DEVELOPER
https://imaris.oxinst.com/big-data	SOURCE LINK

Software	
Imaris File Converter	NAME
Bitplane	DEVELOPER
https://viewer.imaris.com/download/ImarisFileConverter9_9_1w64.exe	SOURCE LINK

Software	
Imaris Stitcher	NAME
Bitplane	DEVELOPER
https://imaris.oxinst.com/products/imaris-stitcher	SOURCE LINK

Data preprocessing

- 1 Convert image stack (.OME-TIFF) to Imaris file type (.IMS) using Imaris File Converter.

Software

Imaris File Converter

NAME

Bitplane

DEVELOPER

https://viewer.imaris.com/download/ImarisFileConverter9_9_1w64.exe

SOURCE LINK

- 2 For mosaic/tile scan acquisitions, stitch the individual .IMS files with Imaris Sticher.

Software

Imaris Sticher

NAME

Bitplane

DEVELOPER

<https://imaris.oxinst.com/products/imaris-sticher>

SOURCE LINK

Spinal cord visualization and segment localization

- 3 Open the final, stitched .IMS file in Imaris.

Software

Imaris

NAME

Bitplane

DEVELOPER

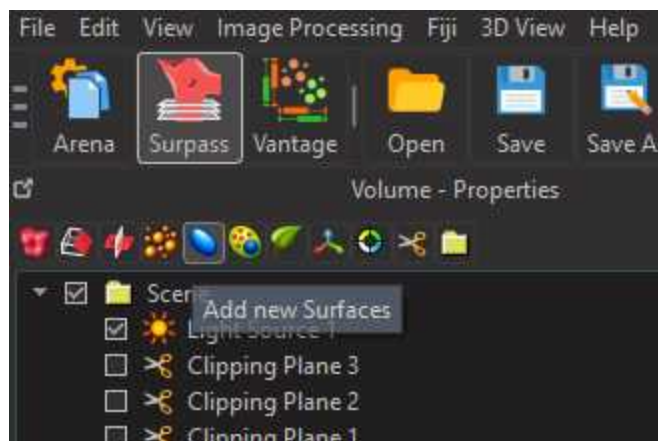
<https://imaris.oxinst.com/big-data>

SOURCE LINK

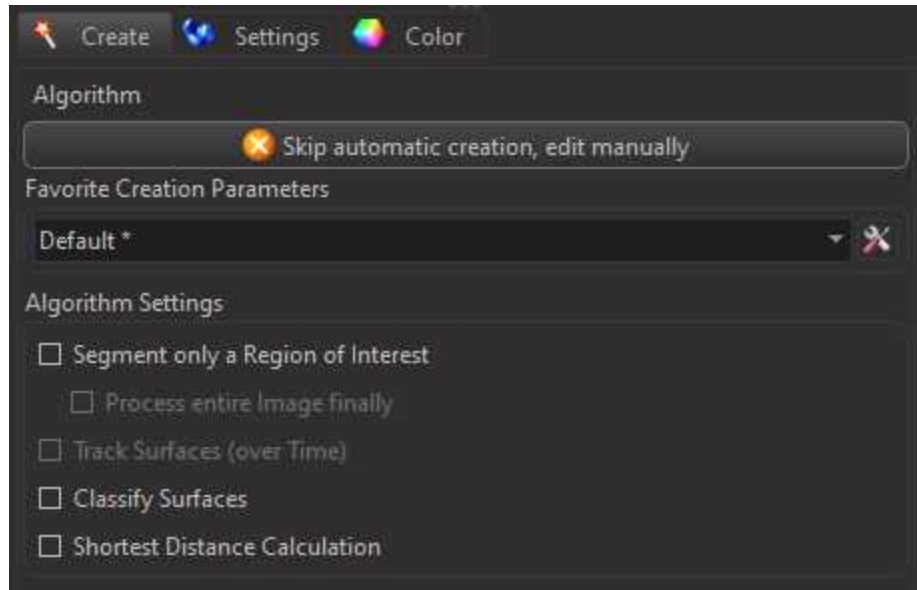
- 4 Using the Orthogonal Slicer in Imaris, optimize the display properties of each relevant channel, focusing the slicer on the motor neurons of interest while doing so. This will typically involve changing the minimum and maximum display and increasing the gamma correction to 1.2 to 1.6.
- 5 Identify spinal cord segment boundaries with the following fiduciary markers:
 - ventral and/or dorsal roots
 - segment-specific cytoarchitectureMark these segment boundaries with transverse orientated Orthogonal Slicers.

Motor pool 3D segmentation

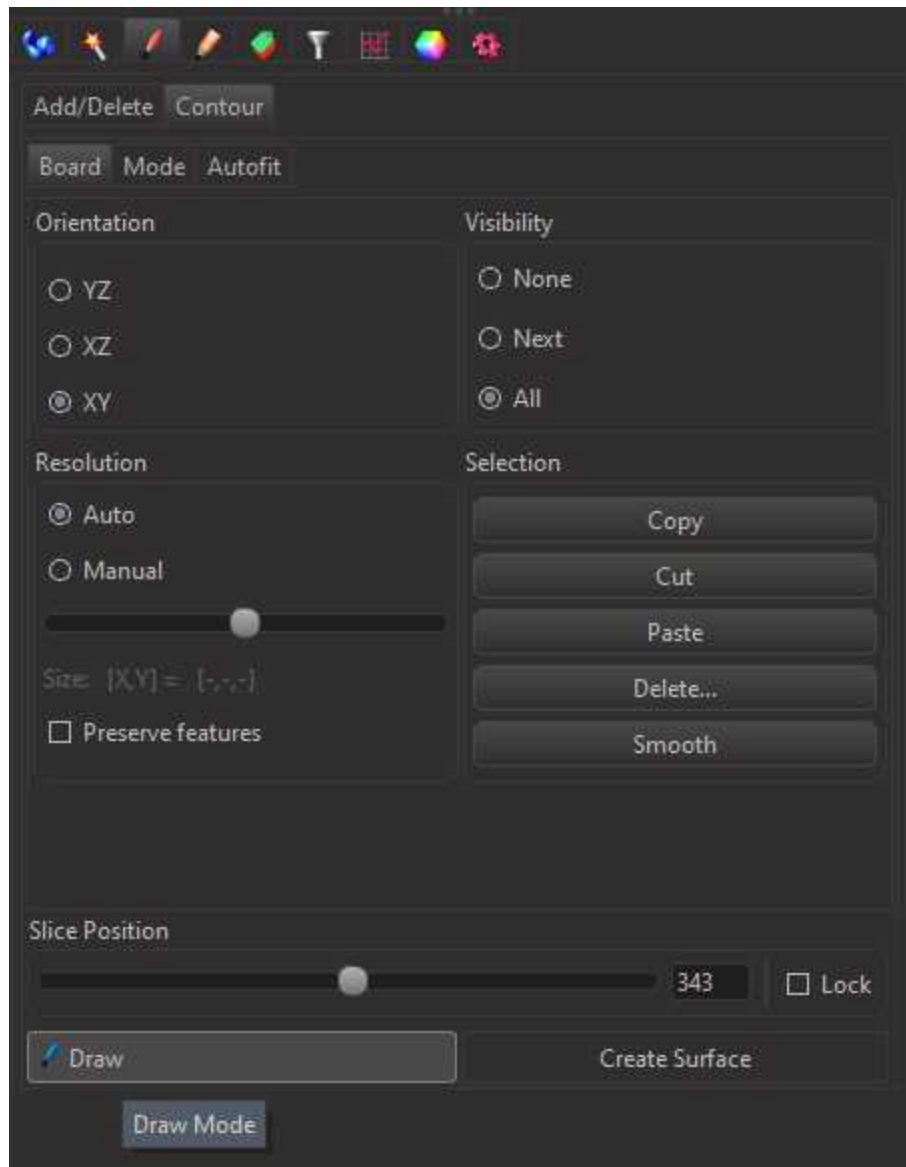
- 6 In Imaris, create a *Surface* by clicking on the blue Surface icon.



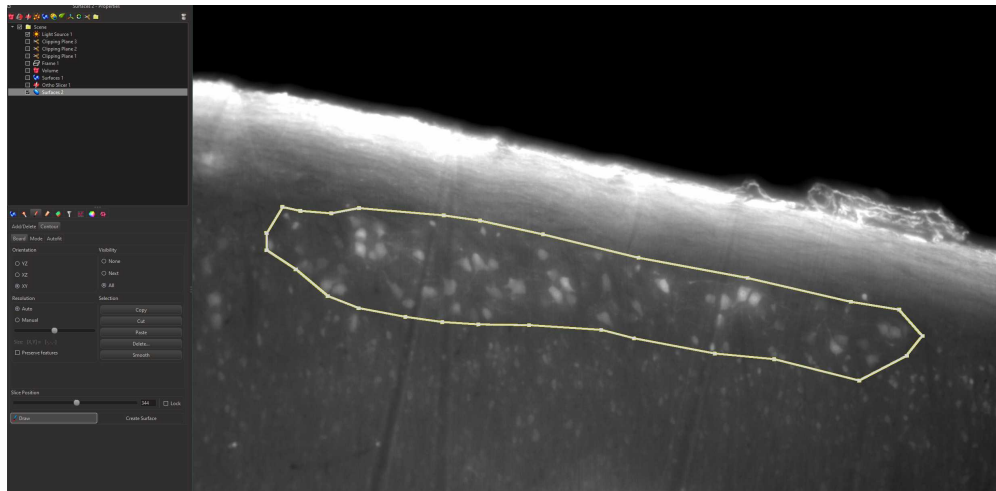
- 7 Click on the large button with the "X" symbol - "Skip automatic creation, edit manually"



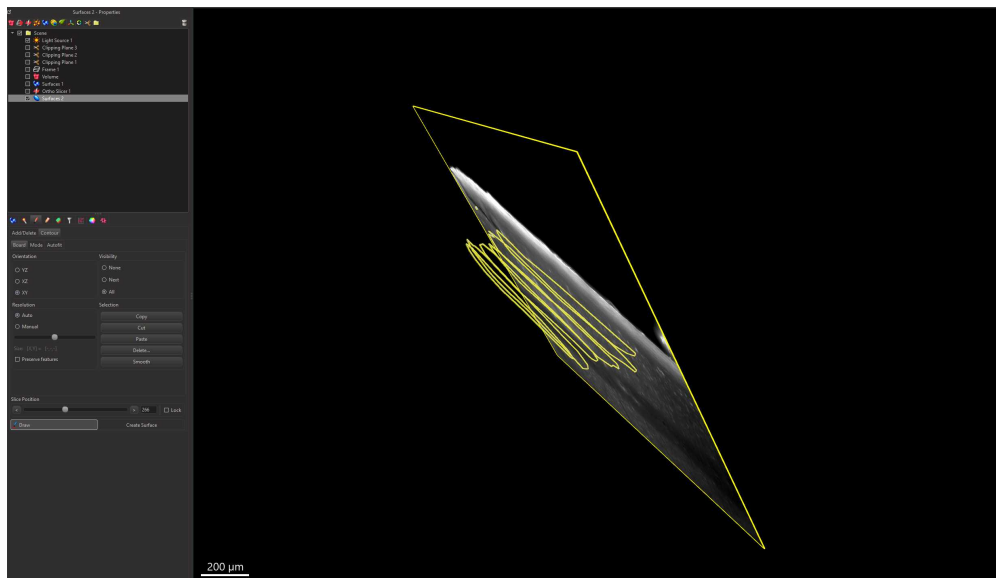
- 8 In the *Draw* tab of the *Surfaces*, go to *Contour* sub-tab and select the *Orientation* of orthogonal slice on which contours will be drawn. The best resolution for light sheet images is always in XY, and was chosen for this example, which is the horizontal/longitudinal orientation relative to the spinal cord.



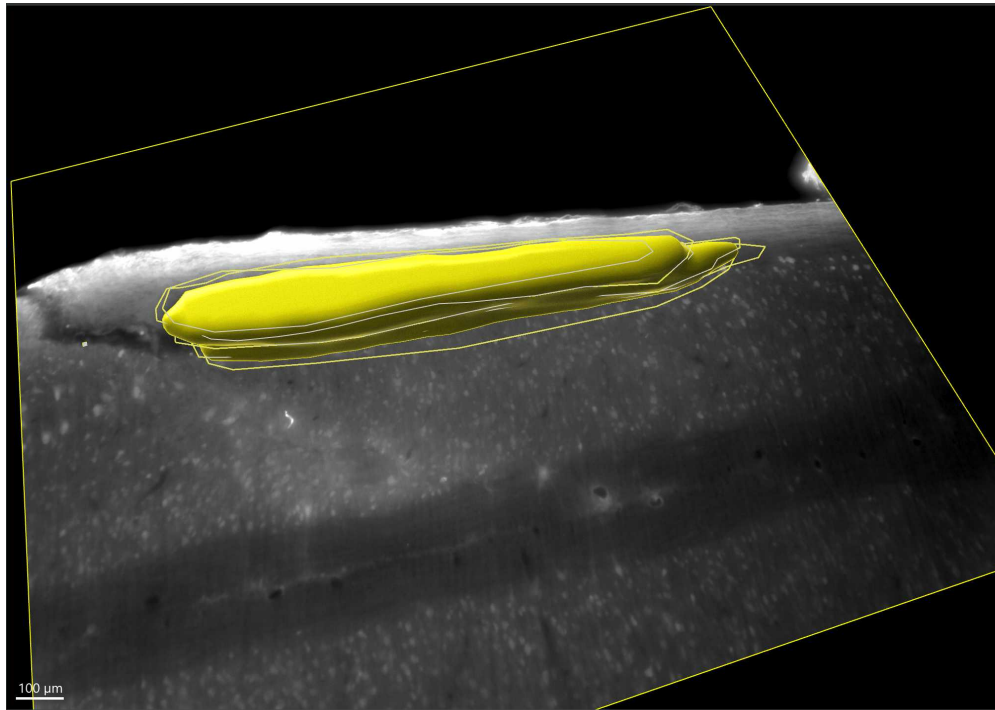
- 9 Move the orthogonal slice to the desired position in the spinal cord i.e. beginning of the motor pool to be segmented.
- 10 Click *Draw* to enable the creation of the contour on the orthogonal slice.
- 11 Create a polygonal contour outline on the orthogonal slice, clicking once to lay down each vertex. To close the contour, simply click on the starting point.



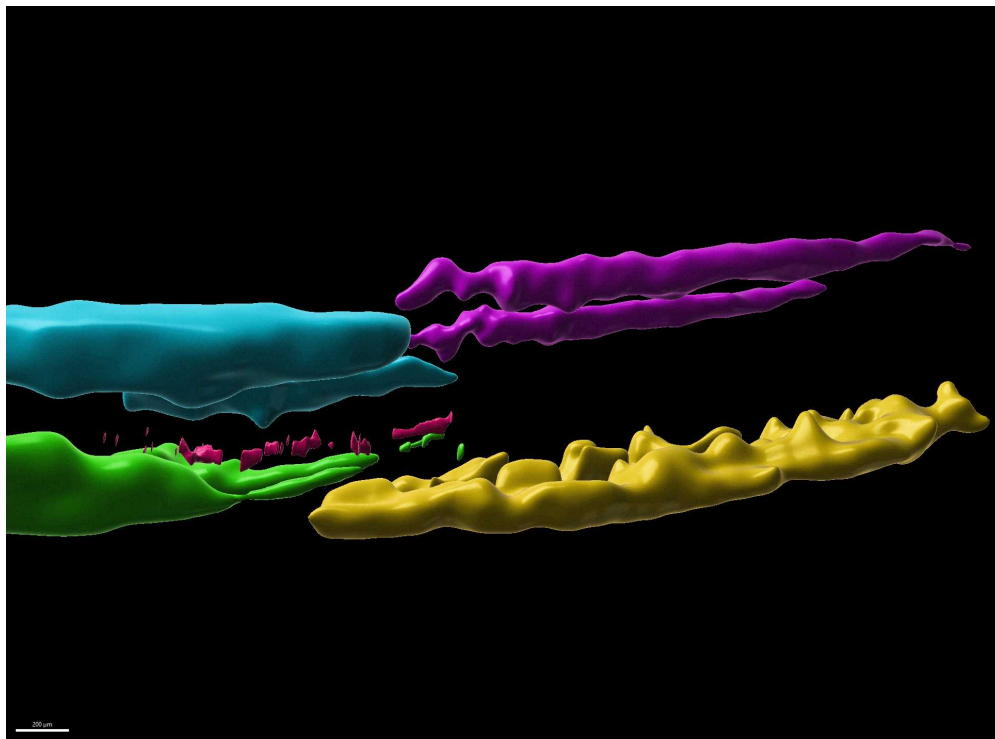
- 12 Repeat the drawing of contours multiple times at different levels, going from the start of the motor pool to the end. The frequency of contours required depends on how detailed the final segmentation needs to be. For exact outlines of neurons, a high number of contours is recommended. In our example, we traced loosely around the motor pool, and hence a low number of contours was required.



- 13 When all contours are drawn for the motor pool, click on *Create Surface* in the *Contour* sub-tab of the *Draw* tab to create the 3D surface.



- 14 Repeat Steps 6 to 13 for each motor pool or spinal cord nuclei to be segmented.



Example collection of segmented motor pools using Imaris Surfaces.



Protocol references

Citation

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<https://doi.org/10.1002/cne.901920313>