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SPARC_Duke_PelotGrill_OT2-OD025340_PigVagusNerve_Morphology

Nicole A. Pelot[1], Gabriel B. Goldhagen[1], Jake E. Cariello[1], Warren M. Grill[1]

[1]Duke University

1 Works for me dx.doi.org/10.17504/protocols.io.6bvhan6

SPARC
Tech. support email: info@neuinfo.org

Nicole Pelot
Duke University

ABSTRACT

The protocol describes image segmentation and image analysis methods to quantify pig vagus nerve morphology from Masson's trichrome-stained nerve cross sections.

DOI

dx.doi.org/10.17504/protocols.io.6bvhan6

PROTOCOL CITATION

Nicole A. Pelot, Gabriel B. Goldhagen, Jake E. Cariello, Warren M. Grill 2020. SPARC_Duke_PelotGrill_OT2-OD025340_PigVagusNerve_Morphology. **protocols.io** dx.doi.org/10.17504/protocols.io.6bvhan6

KEYWORDS

Vagus nerve, nerve morphology, pig vagus nerve, endoneurium, perineurium, epineurium, fascicles, image segmentation

CREATED

Aug 09, 2019

LAST MODIFIED

Jun 30, 2020

PROTOCOL INTEGER ID

26709

MATERIALS TEXT

- Nikon's NIS Elements
- Matlab

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Citation: Nicole A. Pelot, Gabriel B. Goldhagen, Jake E. Cariello, Warren M. Grill (06/30/2020). SPARC_Duke_PelotGrill_OT2-OD025340_PigVagusNerve_Morphology. https://dx.doi.org/10.17504/protocols.io.6bvhan6

Image Segmentation

1 We used Nikon's NIS Elements software (v5.02.01, Build 1270) to segment pig vagus nerve micrographs stained with Masson's trichrome using the General Analysis RGB tool.

2 For each image, we selected preprocessing steps, such as smoothing and sharpening.

3 For each image, we selected ranges of hues, saturations, and intensities to values that identify the endoneurium (fascicles) and different values to identify the entirety of the nerve.

4 For each image, we selected postprocessing steps, such as setting a minimum size criterion (eliminate small off-target regions), smoothing, cleaning, closing, and filling holes.

5 We made manual adjustments as needed, including manual deletion of off-target regions and filling of target areas that had not been captured.

6 We converted the binary segmented image into “Graticule Masks”, binary images saved as TIFs.

Image Analysis

7 We imported the TIFs into Matlab and generated a data structure of the x and y coordinates of the pixels for each closed boundary of the loaded binary images using the *bwboundaries* function.

8 We scaled the pixel coordinates to microns using the segmented scale bar.

9 We calculated cross-sectional area of each fascicle and nerve using Matlab’s polyarea. Effective diameter (for a nerve or fascicle) is the diameter of the circle that has the same cross-sectional area as the raw trace.

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