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SPARC_Duke_PelotGrill_OT2-OD025340_RatVagusNerve_Morphology

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1 Works for me dx.doi.org/10.17504/protocols.io.y6hfzb6

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ABSTRACT

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

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KEYWORDS

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

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MATERIALS TEXT

- Nikon's NIS Elements
- Matlab

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Image Segmentation

1 We used Nikon's NIS Elements software (v5.02.01, Build 1270) to segment rat 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 perineurium and, in multifascicular cases, different values to identify the entirety of the nerve. For the cervical samples, we identified the vagus as the largest nerve in the carotid sheath. For the subdiaphragmatic samples, we identified and segmented the largest fascicle on the anterior surface of the esophagus; we also segmented smaller fascicles nearby if they were approximately within double the diameter of the largest fascicle in distance from the edge of the largest fascicle. For monofascicular nerves, the outer perineurial boundary was used as the nerve boundary. For multifascicular nerves, the nerve trace was identified as the approximate transition from denser connective tissue grouping the fascicles to looser connective tissue.

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 stored the pixel coordinates with indexing that assigns a fascicle number, which were then checked so that both the interior and exterior perineurium trace relate to the same fascicle.

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

10 We calculated cross-sectional area of each fascicle (inner perineurium and outer perineurium traces) 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. The perineurium thickness is half of the difference in effective diameters of the inner and outer perineurium traces.

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