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SPARC_Duke_PelotGrill_OT2-OD025340_PigVagusNerve_Morphology

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We use this protocol and it's working

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Abstract

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

Materials

- Nikon's NIS Elements
- Matlab

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.