Blob Diameter & Ring Thickness: Application to measure axons and their myelin sheath.

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Neural interfaces enable reading from and writing into the peripheral nervous system. However, incomplete characterization of the long-term usability and bio-integration of intra-neural electrodes have restricted their clinical use. Aiming at filling this gap, we assessed the impact of the implanted electrodes on the nerve by measuring classical markers of nerve “quality”, i.e. the diameter of the fibers composing the nerve and the thickness of their myelin sheath, and analyzed them with respect to their distance from the electrode and as a function of implantation time.

From a image processing perspective the main challenges of this project are:

i) the heterogeneity within the image, ii) the size of the images, iii) the total number of objects, iv) the variability of object diameters, v) the number of measurements.

**From original image to the output**

**Line Profile and Fitting**

Using a previously developed tool in the “Multi-Dimensional Gabor” (mDoG) custom Application of ImageJ (ROI) the user defines the fascicles and the electrodes. Then the ImageJ macro takes advantage of the Parallel Processing plugin to analyse the image.

**Multi-Difference of Gaussian (mDoG)**

To accurately detect the local maxima, we generated a multi-Diff-Gaussian image (A) and used the function “Find Maxima” with a noise tolerance of 20. Because the axons one could vary a lot this method appeared to be much more efficient than using a single Gaussian Blur or a channel.

A “classic” DoG image is obtained by subtracting the image convolved with the Gaussian of variance σ2 from the image convolved with a Gaussian of smaller variance σ1 (i.e. σ2 > σ1).

The multi-Diff-Gaussian image obtained by subtracting the image convolved with the Gaussian of variance σ1 from the image convolved with a Gaussian of smaller variance σ1 (i.e. σ2 > σ1).

**Threshold Distance Map Measure**

To measure the distance of the closest neighbor. The super-gaussian formula allows to fit a variety of shapes of the signal and to accurately measure the full width at the maximum.

**Neighbour map**

The points detected on the mDoG image, were drawn on a new image and the corresponding threshold distance was calculated, before being thresholded. This binary image was finally used to make a distance map. Measuring the value at a point on this image gives the closest distance to a neighbouring edge. This value is then used for the line profile measurement and fitting map.

**Selected solutions:**

i) analyse the entire image, ii) downsample when possible, iii) use curve fitting, iv) use multi-thread plugin (parallel processing).

**WorkFlow**

Analysis was carried out on implanted nerve cross-sections stained to reveal axons and their myelin sheath. The tiled image of the stained nerve section was acquired on a wide-field microscope, and contained between 1000 and 4000 myelinated and unmyelinated axons per image, distributed in different regions of interest (ROIs).

(A) The tiled image of the nerve section stained for beta-tubulin (in red, corresponding to the axon and for NF-H (in green, corresponding to the myelin). The white square corresponds to the magnified area showed in the following panels. (B) On the axes channel (five UX), the ROIs corresponding to the three fascicles contained in the rat sciatic nerve (green) and to the implanted electrodes, (C) The white (at points of the ROI corresponding to the outside of the fascicles used to measure the average intensity of the axon channel, defines the baseline). (D) From the image channel 4 ROIs (E) The “Axon fitted” are represented in red with the calculated diameter, “Axon unfitted” and “Axon too close” are represented as circles with the calculated diameter, “Axon un-fitted” and “Axon too close” are represented as circles with the calculated diameter. (F) The “Axon fitted” are represented in red with the calculated diameter, “Axon unfitted” and “Axon too close” are represented as circles with the calculated diameter.

Finally, the image series was stacked and normalized by their standard deviation (σ1) [2]. To accurately detect the local maxima, we generated a multi-Diff-Gaussian image (A) and used the function “Find Maxima” with a noise tolerance of 20. Because the axons one could vary a lot this method appeared to be much more efficient than using a single Gaussian Blur or a channel.

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The multi-Diff-Gaussian image obtained by subtracting the image convolved with the Gaussian of variance σ1 from the image convolved with a Gaussian of smaller variance σ1 (i.e. σ2 > σ1).

**References**

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4 Under development.