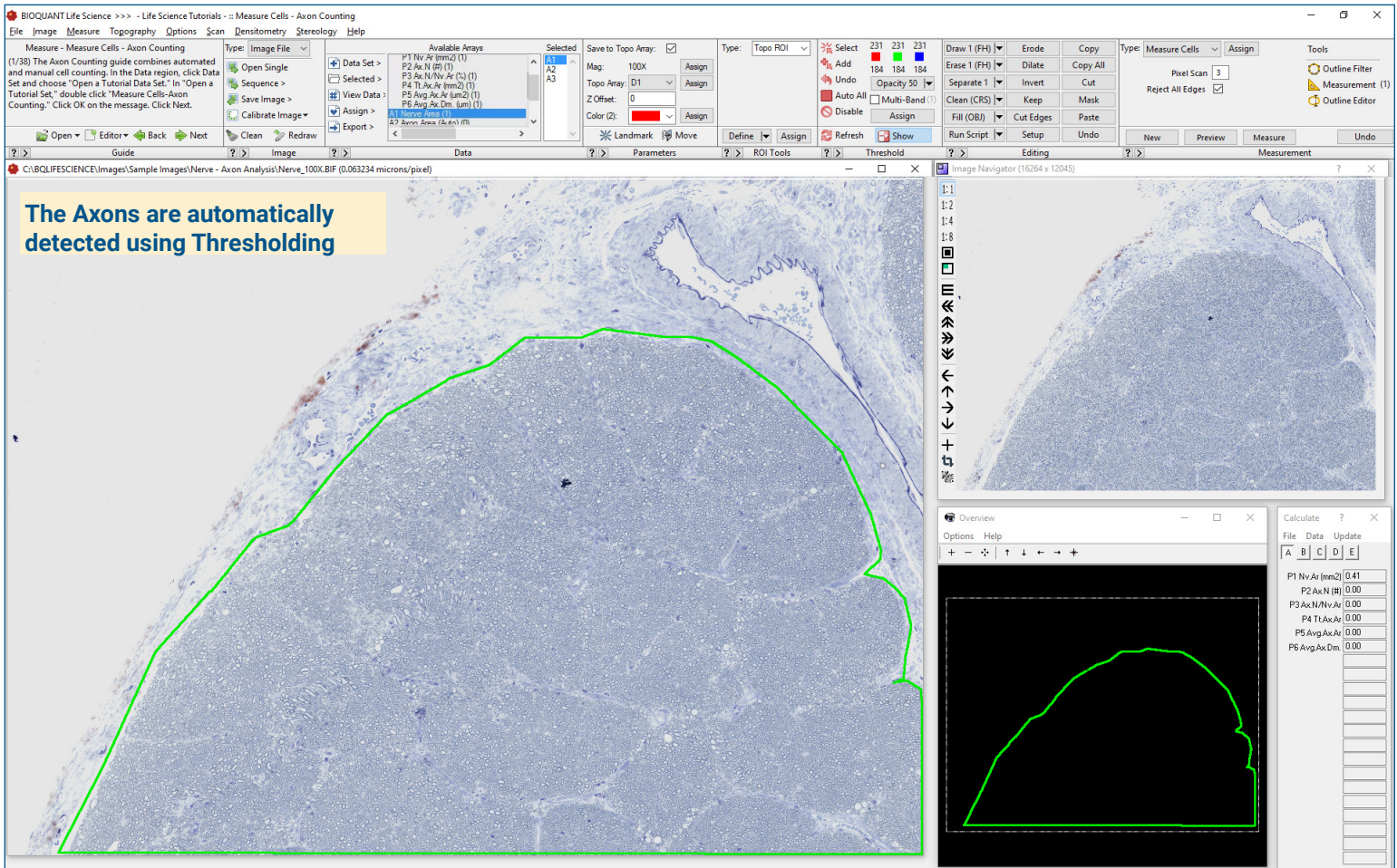


Axon Counting Protocol



Get Image of the Section

Up to **4 GB Scan** of Section:

- Scans can be generated manually by BIOQUANT Life Science, automatically by BIOQUANT SCAN, or via a third party slide scanner.
- In BIOQUANT, zoom out so the entire Sampling Area fits in one field of view.

Live Image from Microscope with Camera and tracking device:

- In BIOQUANT, see a live image of the tissue directly in the Image Window at a low objective like 4X or 2X.
- The Sampling Area does not have to fit in one field of view.

Define Nerve Sampling Area

A Sampling area defines a region at low power on the section within which cells are measured at high power.

Define the nerve borders using the **Irregular Region of Interest** tool.

- BIOQUANT limits threshold to within the ROI and collects Nerve Area data
- If more than one field of view is required, BIOQUANT automatically reports the total area within the region of interest for both fields.

Zoom In or Change Objective

Digital Scan in **Large Image Navigator**:

- Zoom in to higher magnification.
- Pan to the upper left corner of the Sampling Area. **Auto ROI** always keeps you within the Sampling Area.

Live Image with Microscope:

- Change to a higher objective
- Move to the upper left corner of the Sampling Area.
- The Sampling Area tracing redraws on the live image.

Axon Counting Protocol

Counting Axons Field to Field.
Previously measured axons are automatically rejected.

Preview Axon Data

Click **Preview** to automatically:

- **Threshold** the axons
- Preview outline the axons, using **shape and area filters** to remove false positives..
- Axons already measured on previous, overlapping fields of view are **not** preview outlined.
- Axons cut by the region of interest are **not** preview outlined.

Refine & Measure Axon Data

You can refine the preview outlines, if necessary:

- Use **Draw Threshold** to define axons that did not threshold.
- Use and **Erase Threshold** to remove false positive axons.
- The Preview outlines update with the changes.

Click **Measure** to record Axon data.

Repeat for all fields in the Sampling Area.

Data Collection

Computed Data:

- Nerve Area
- Individual Axon Area
- Individual Axon Caliber
- Axon Count
- Axon Density (#/unit area)
- Mean Axon Area
- Mean Axon Caliber

This is a sample protocol. It is easily modified for different animals, tissues, embedding methods, and stains. All parameters can be renamed. New calculations can be added. Both live imaging and scans are supported. Measure at the magnification that is best for your histology. Find out more: www.bioquant.com/lifescience