Neuronalyzer - User Manual

The software is compatible with MATLAB R2020b or newer.

To get started, download the latest Neuronalyzer version from the GitHub repository:

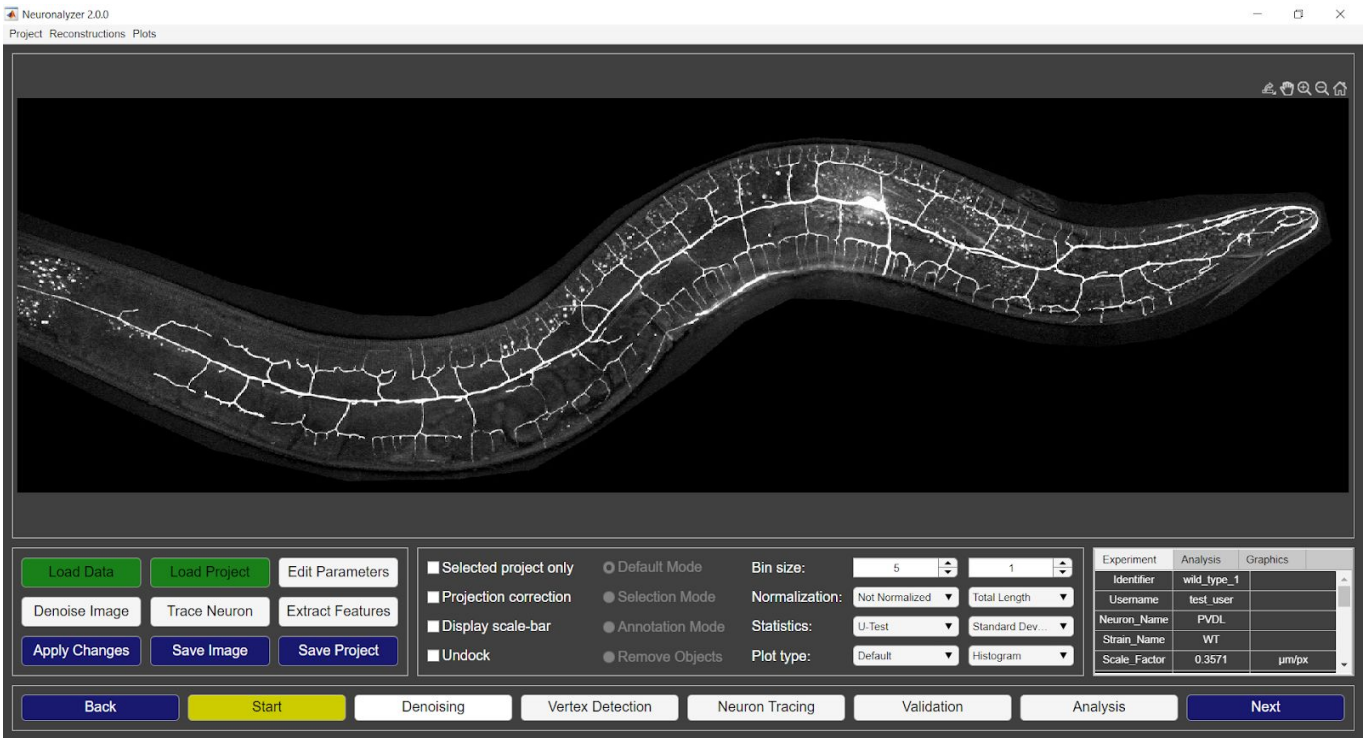
https://github.com/Omer1Yuval1/Neuronalyzer

Then open the file index.m in MATLAB and run it.

1. Load an image or an existing project

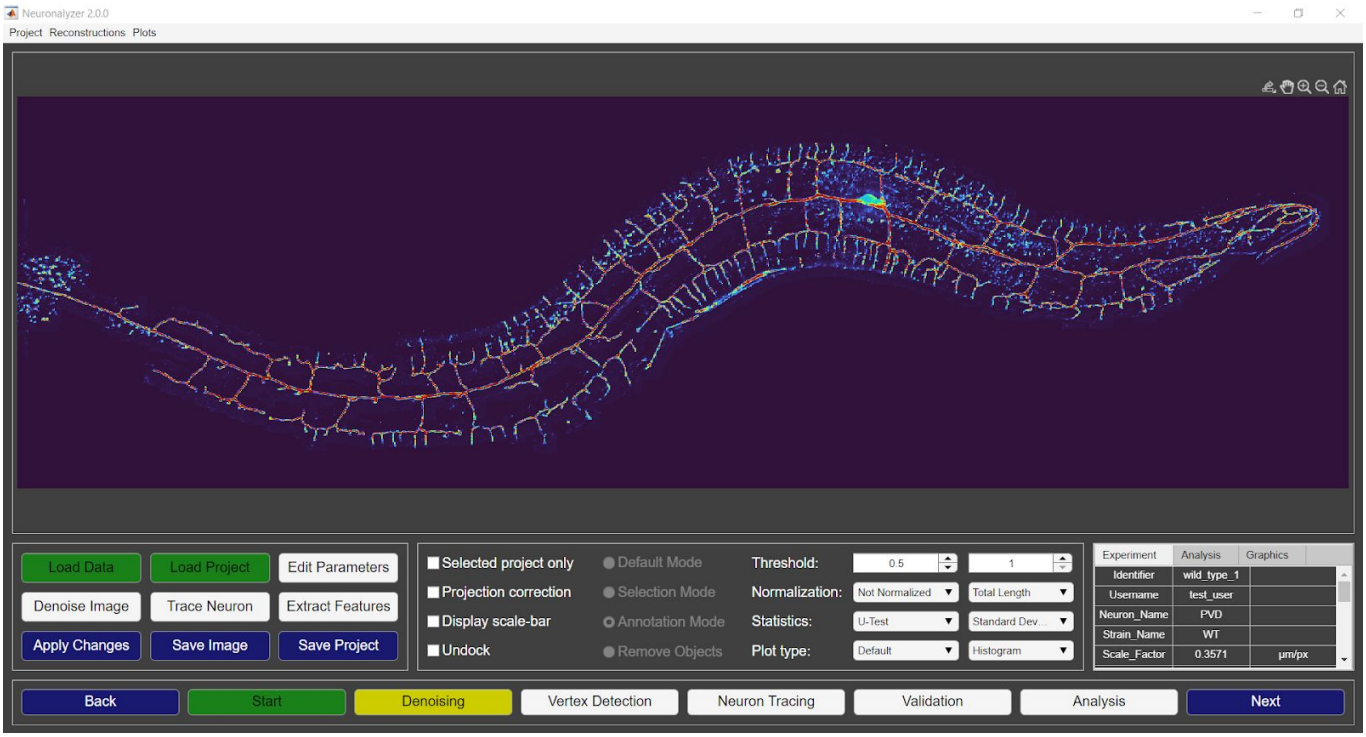
Use the “Load Data” button to load an image(s), or the “Load Project” button to load an existing project file(s). Then, use the “Project” menu to navigate between the different images/projects. You can load multiple image/project files at once, or load a project file that contains multiple projects.

Use the tables at the bottom-right corner to add meta-data. The first table allows you to enter experimental information such as scale-bar, temperature and strain name (the third column is used for units). The second table allows you to enter analysis information for reproducibility. This includes the version and commit id of the code used for the analysis, as well as the date and the name of the person that performed the analysis. You can find the commit id on the GitHub repository (for example: 357b6ac).



2. Denoising

Next, you can use the “Denoise Image” button to apply a denoising neural network. This will increase the contrast between neuron and non-neuron pixels using a pre-trained convolutional neural network (CNN). You can adjust the threshold and minimum object size. These will be used to threshold the denoised image and remove small objects (respectively), resulting in a binary (black & white) image (see next step). To display the denoised image, in the “Reconstructions” menu, choose CNN → CNN Image - RGB.



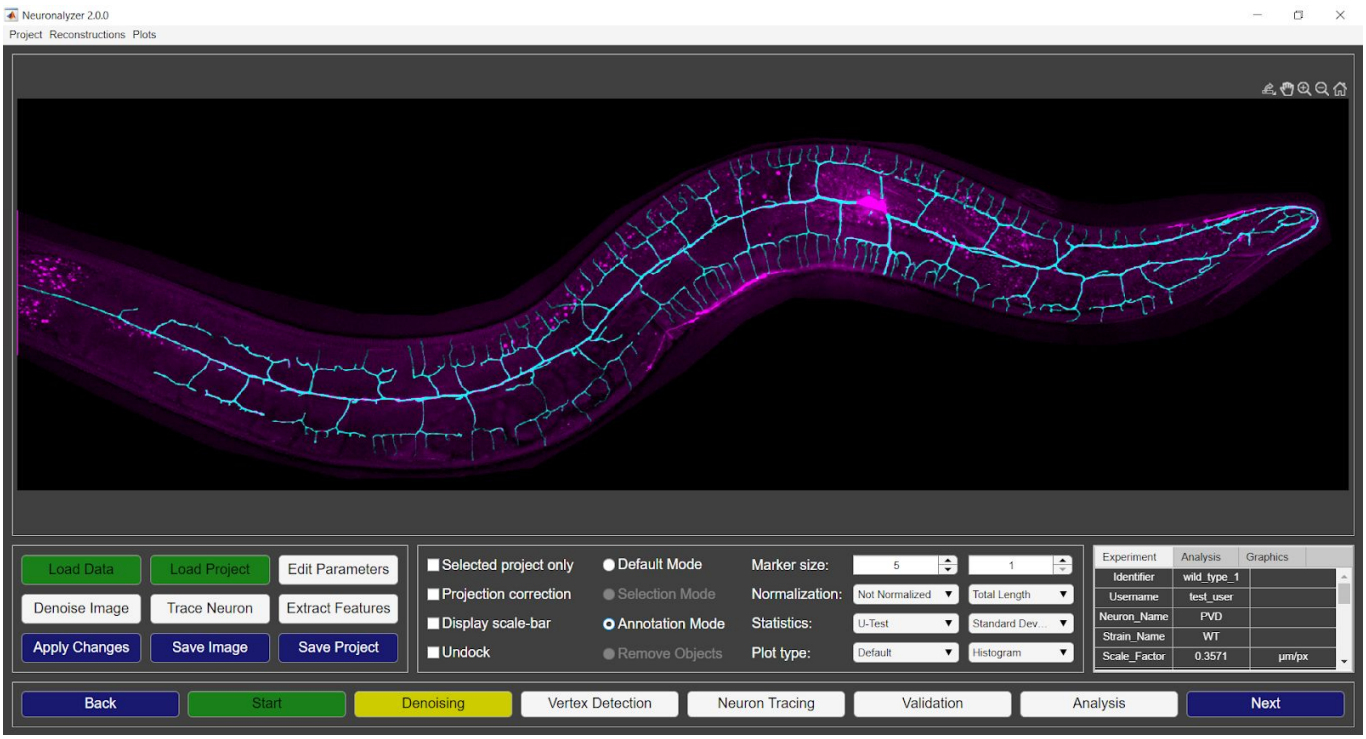
3. Manual annotation

Once the image is denoised, you can display the resulting binary (black & white) image from the “Reconstructions” menu (Binary Image → Binary Image). This image will be used for the mapping of neuronal junctions, as well as for neuron tracing.

You can also display this image using a colormap for visual and annotation purposes (see below), where “White” (1) and “Black” (0) are displayed using their intensities in the original grayscale image.

You can manually edit the binary image by adding or removing pixels. First, zoom in to magnify a certain image region and choose a marker size. Then, choose “Annotation Mode”. You can then use the mouse to add (left-click) or remove (right-click) pixels. Once finished, change back to “Default Mode”. The result is automatically saved.

Note that if you repeat the previous step (apply the image denoising operation), this will overwrite your binary image and annotations. You can save your work at any time using the “Save Project” button.



4. Neuron tracing

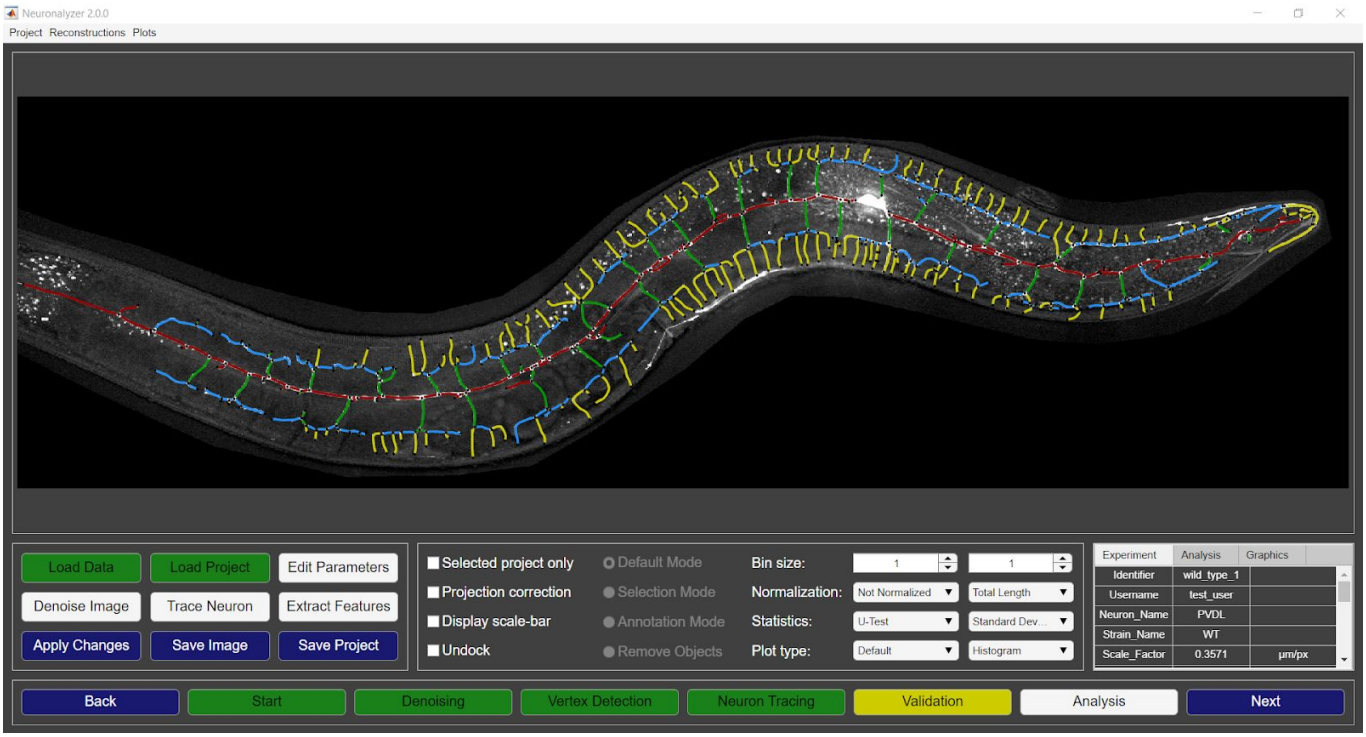
To obtain the trace of the neurons, click the “Trace Neuron” button. Once finished, the resulting trace is displayed. You can use the “Project” menu to navigate between the different traced images.

5. Neuron axes

You can use the annotation mode to display interactive points that can be moved around to tweak the axes positions (Reconstructions → Axes). Use the spinner to specify the number of interactive points (here set to 25 points), then click the “Apply Changes” button.

6. Validation

Once the images have been traced, you can use the “Reconstructions” menu to visualize various morphological features. The examples below show the radial distance from the midline (top), and the classification of neuronal elements into four morphological classes (bottom).



7. Analysis

Finally, use the “Plots” menu to display quantifications of the extracted features. Use the control panel to specify plot parameters, then click “Apply Changes”. Plot parameters include bin-size, normalization, statistics and plot types. The examples below show the mean and standard deviation of neuronal length per morphological class for each group of animals (top), and the density of neuronal elements along the neuron’s midline, averaged across wild-type animals (bottom).