NeuroPAL ID Software Instructions

Capturing Appropriate, High-Quality Images

1. Please read the 2 NeuroPAL manuals on configuring your microscope and taking good pictures:
    A) README 1st - Quick Start Guide to NeuroPAL
    B) Configuring Your Microscope for NeuroPAL
2. Ensure your images look similar to those in the manuals:
    A) Bright colors across all channels.
    B) High resolution.
    C) Cropped to maximize the worm and minimize any empty background.
3. We are encouraged by your faith, but this software cannot work miracles for poor quality images.

Opening Your Image

1. Use the menu **File|Open** to open your image.
2. Ease your mind, the software auto-saves every change you make in all steps below.
3. Enter info for the image: **Body**, **Age**, **Sex**, **Strain**, and optional **Notes**.

*Note 1: you must specify **Body**, **Age**, and **Sex** for automated ID.*

4. If incorrect, set the dropdown **Colors: R**, **G**, **B**, & **W** channels to reflect the red, green, blue, and white (panneuronal) NeuroPAL color configuration.
5. Optional: set the dropdown **GFP** channel to reflect your reporter if you have one.
6. Optional: use the menu **Image|Rotate All** to orient the image however you desire.

Note: publication convention is left=anterior & top=dorsal.

7. Use the user interface under the image to indicate the worm orientation:
    A) Set the dropdown to either **L/R** (Left/Right) or **D/V** (Dorsal/Ventral).
    B) Press the button **Flip Z** to ensure the Z slider reflects the appropriate orientation for the Z slices. The image color indicates left/dorsal Z slices (cyan), center (gray), or right/ventral Z slices (magenta).
    C) Set the field **Center Z** to roughly reflect the central Z slice for the worm.

The Best & Fastest Way to Use the Software

1. Most people want to quickly annotate their images for either of the following reasons:
    A) Identify and quantify reporter expression (GFP/CFP/YFP).
    B) Quantify neuron positions.
    C) Whole-brain activity imaging (GCaMP) which is, unfortunately, **NOT YET AVAILABLE**.
2. For A & B, the neurons of interest are often only a small subset that need to be annotated.
3. Mark your neurons of interest by double-clicking on the image to add or remove neuron dots.
4. Use the manual to identify your neurons of interest:
    A) Type their name in the **User** field, then press the space bar or **User ID**.
    B) Or, choose their names from dropdown **Auto**, then press **Auto ID**.
5. Press the **Save: IDs** button to save all information to a spreadsheet.

Automated Neural Identification

1. Superficially, automated neural identification sounds like the easiest method. But:
    A) Your images MUST be of good quality.
    B) You will now need to ensure ALL and ONLY neurons are marked in the body region.
    C) You will still need to review the neural identities assigned by the software.
    D) If the software guessed wrong, you will need to correct it and re-run the auto ID.
    E) Therefore, use this method only when you need to identify many neurons.
2. Optional: use the menu **Preprocessing|Artifact Removal** to remove artifacts that might be confused with neurons (e.g., gut granules & hypodermal nuclei).
3. Press the button **Auto-Detect** to find the neurons in the image.
4. Optional: double-click on the image to add or remove neuron dots.
5. Press **Auto-ID All** to auto ID all neurons in the image.
6. Review IDs for the top ~5 neurons in the list of **Neuron Ranked Confidence**:
    A) Click the top items in the list.
    B) Optional: accept the ID by pressing either the space bar, **Auto ID**, or **User ID**.
    C) Optional: correct the ID by either choosing it from the dropdown **Auto** and pressing **Auto ID** or typing it into **User** and the space bar or **User ID**.
    D) Optional: if you are unsure of an ID, choose a different neuron in the list to ID instead.
7. Optional: if you are unsure of an ID but wish to include it, mark it as low confidence by adding a “?” at the end in the **User ID** field.
8. Optional: neurons can be labeled as an unspecified “ARTIFACT”, “MUTATION”, or emphasized by adding a “!” at the end in the User ID field (e.g., to emphasize neuron duplications in mutants).
9. Optional: delete your IDs by replacing the name in the **User ID** field with a space.
10. Optional: press **Auto-ID All** at any time after ID’ing additional neurons to improve the automated ID results for the image.
11. Optional: perform #4 & #5, at your leisure, to add/remove neurons and re-auto-ID.
12. ID as many neurons as you need in the image.
13. Optional: use the menu **Analysis|Save ID Image** to save your image as slices with your neuron IDs superimposed. These PDF files have the same name as your image and are saved in the same folder.
14. Optional: press the **Save: IDs** button to save all information to a spreadsheet. The spreadsheet has the same name as your image, in the same folder, and ends in “.csv” (compatible with all popular spreadsheet programs Excel, Numbers, …). The spreadsheet contains appropriate thresholds for your optional reporter listed as background for the “GFP” channel, an Otsu threshold (assuming reporter expression is bimodal, present or absent, across the neurons), and a linear change point at which the GFP intensity markedly increases – we recommend using the change point threshold. “Z-scored” mean and standard deviation are listed per color channel. “Z-scored” colors are roughly comparable across images, but they fail to account for any loss of intensity as the z-plane progresses further from the objective. “Aligned” XYZ positions and RGB colors have been aligned to a statistical atlas and are thus comparable across images as long as the atlas versions (displayed in the spreadsheet) remain the same.