NeuroPAL ID Software Instructions

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.

8. Optional: use the menu **Preprocessing|Artifact Removal** to remove artifacts that might be confused with neurons (e.g., gut granules & hypodermal nuclei).

9. Press the button **Auto-Detect** to find the neurons in the image.

10. Optional: double-click on the image to add or remove neuron dots.

11. Press **Auto-ID All** to auto ID all neurons in the image.

12. 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.

13. 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.

14. Optional: delete your IDs by replacing the name in the **User ID** field with a space.

15. Optional: press **Auto-ID All** at any time after ID'ing additional neurons to improve the automated ID results for the image.

16. Optional: perform #9 & #14, at your leisure, to add/remove neurons and re-auto-ID.

17. ID as many neurons as you need in the image.

18. 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.

19. 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 both background for the "GFP" channel and an Otsu threshold (assuming reporter expression is bimodal, present or absent, across the neurons). "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.