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1. INTRODUCTION

1.1 License and Development

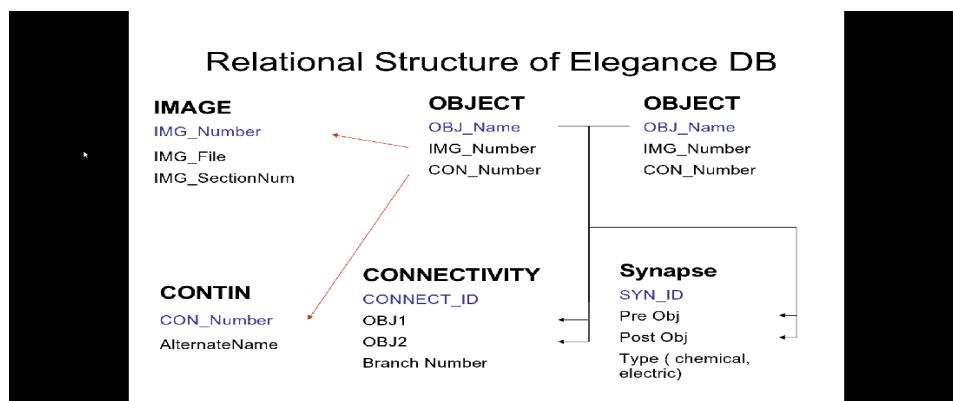
Elegance is open-source software developed by the [Scott Emmons Lab](#). Elegance is free for download and can be found at <http://www.github.com/Emmonslab>. This software is provided with ABSOLUTELY NO WARRANTY and an applicable license can be found at <http://www.gnu.org/licenses/gpl.html>. This software was developed with the help of [Meta-Helix](#) and [Boyle Software](#).

Questions concerning Elegance should be directed to [Steven Cook](#) of the Emmons Lab.

1.2 About

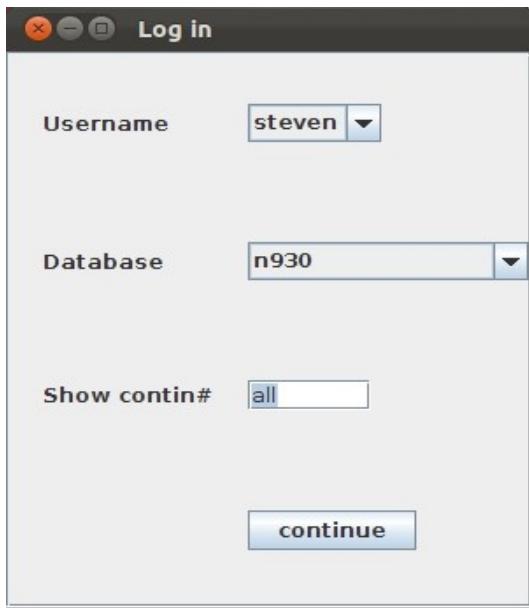
Elegance is a JAVA-based program that facilitates the tracing and reconstruction of neurons across serial section images. The location of a neuron profile in an image is represented by a single point and neuron reconstructions are non-volumetric stick figures. Neuron diagrams created by Elegance show the pathway of the neuron through the tissue or organism and neuron architecture along with the locations of synapses along each branch. Multiple neurons can be traced simultaneously. To reconstruct a neuron, the locations of structures in images, both neurite profiles and synapses, are entered from the computer screen with the mouse. Coordinates and associated attributes are stored in a MySQL database. Relationships across images are stored in a Connectivity Table. From the information in the database tables, Elegance draws 2D or 3D neuron diagrams, generates synapse lists, and adjacency matrices.

To reconstruct a neuron, the user asks Elegance to calculate a “contin.” A contin is a string of connected neuron objects representing a partially or fully reconstructed neuron. Starting from a user selected neuron object, Elegance queries the Connectivity Table and collects all the objects joined directly to the chosen object or indirectly to it via other objects in a continuous chain, moving through the stack in both directions and along all branches until all ends are found. Elegance assigns a unique contin number to this chain (or reassigns the previous number to a recalculated contin). The contin number and a user assignable alternate name (such as a neuron name) are displayed over the lines connecting objects in the contin. A collection of synapse objects on adjacent images which correspond to a single synapse are also considered individual contins by Elegance. Below is a relational structure schematic of the a Elegance MySQL database.



1.3 Login

To open Elegance bash the start script within the Elegance directory.

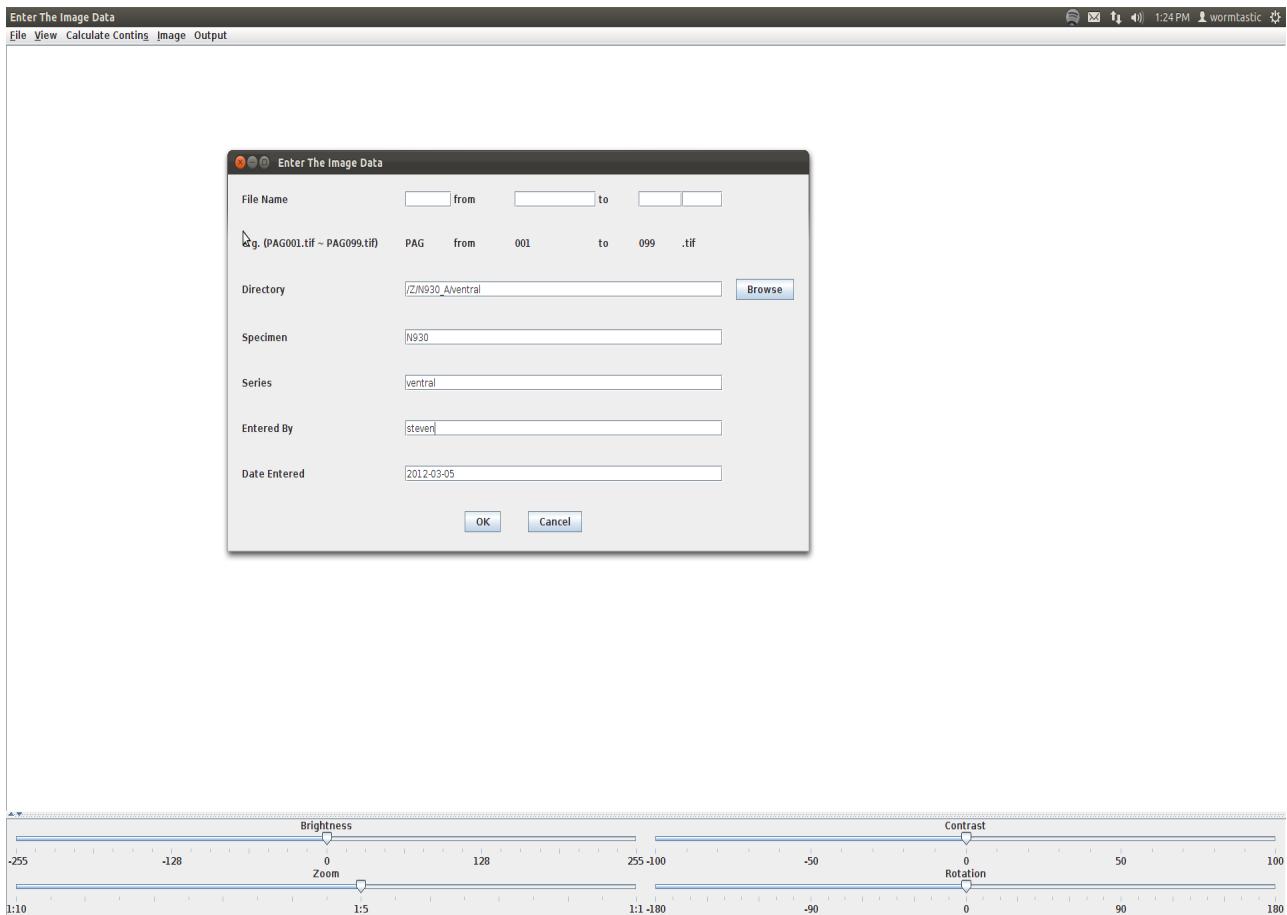


From the login screen you will see options for username, Database, and show contin#. Usernames are entered via the configuration.txt file within the Elegance directory. Usernames can be used to identify who has marked an object. Databases names are entered through the configuration.txt file and should follow the structure of the provided structure.sql file. We suggest using phpMyAdmin to manage your MySQL server. Your configuration.txt file must include your MySQL server address, username, and password.

2. USING THE SYSTEM

2.1 Image Data Entry

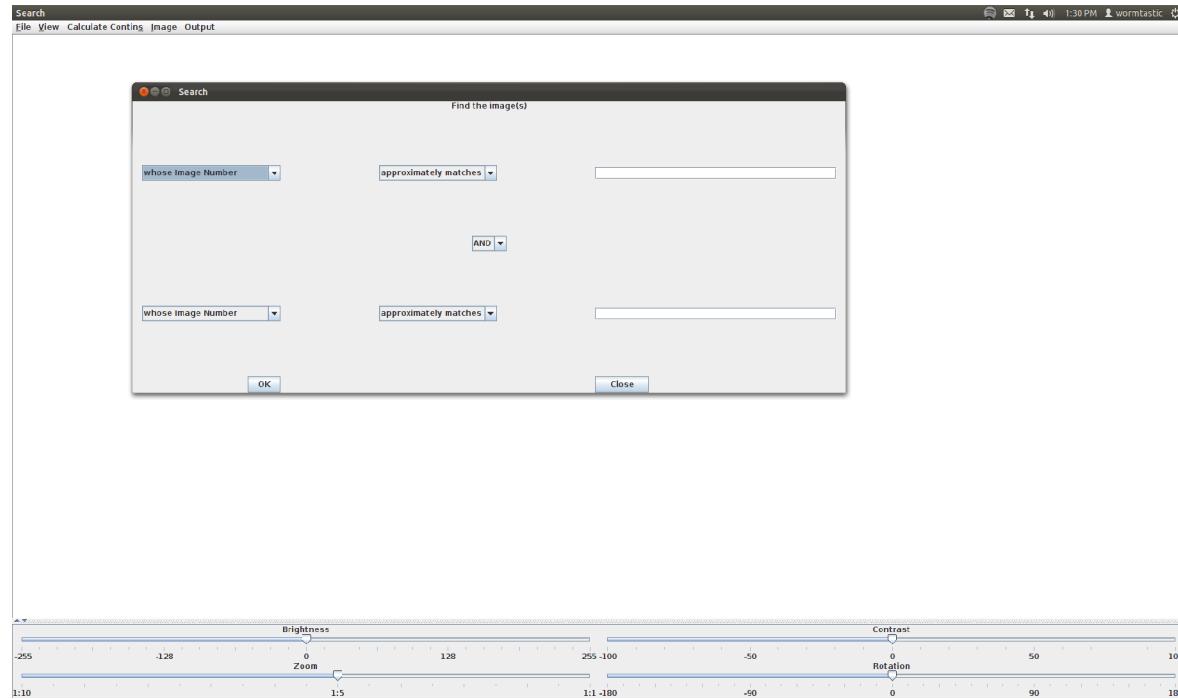
Choose 'Image' -> 'Enter' from the main menu (or press 'Ctrl+E'). The image entry window will pop up as shown below:



The window has text boxes in which information can be entered. Information from the last data entry will be included. Since most of the images will have similar values, data entry can be done easily. The 'browse' button on the window can be pressed to navigate to the location of the image and the image file name & directory path will be filled automatically. Rest all fields must contain some relevant data i.e. can NOT be blank, empty or NULL. On pressing the 'OK' button, the data will be entered into the relational database and a success message or an error message will be displayed. Duplicate image names can not be used. The window can be closed by pressing the cancel button. On 'cancel' or on 'exiting' the window, any data present on the window, if not entered into the database will be lost. Image data entered will be inserted into the 'Image' table in the MySQL database.

2.2 Finding and Loading Images

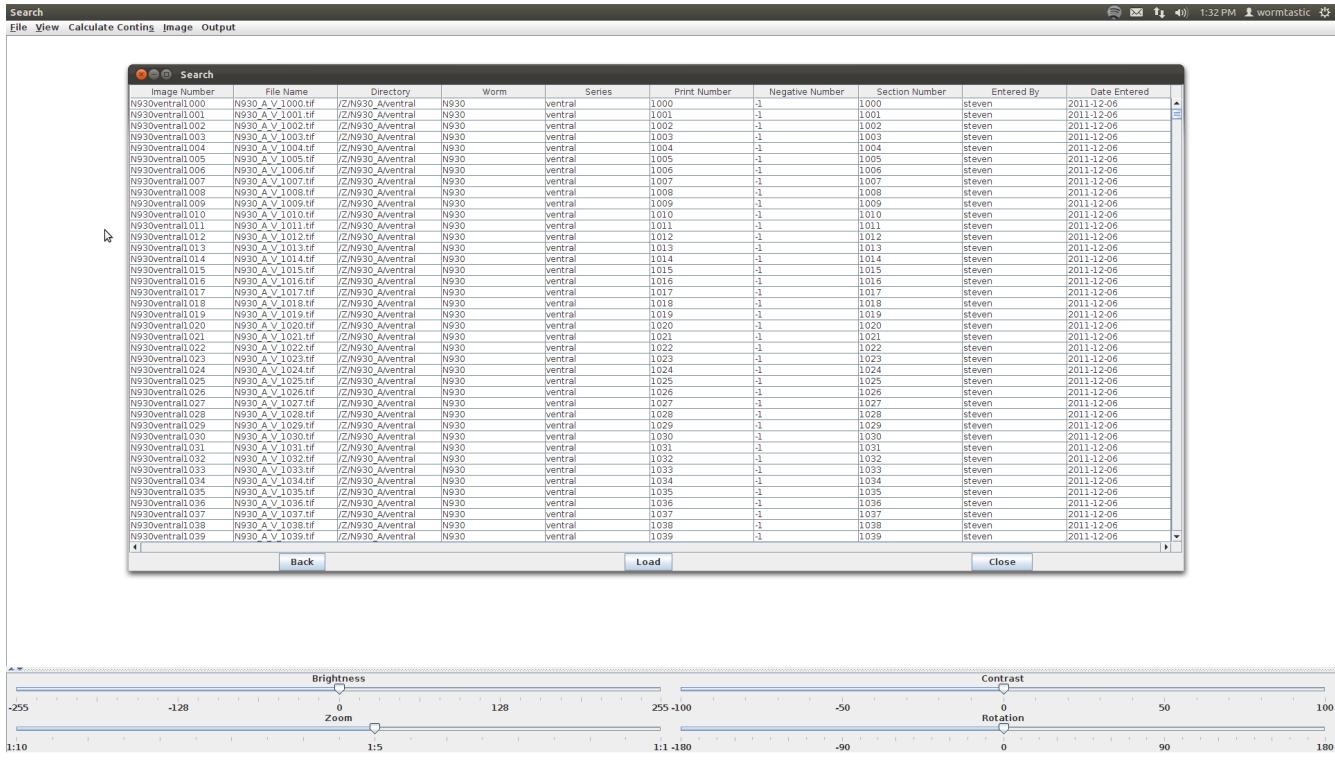
Choose ‘Image’ -> ‘Find’ from the main menu (or press ‘Ctrl+F’). The image search window will pop up as shown below:



Search can be performed using any of the following options: "Image Number", "File Name", "Directory name", "Worm Name", "Series", "Print Number", "Negative Number", "Section Number", "user's name", and the "image entry date". Any of these options can be selected from the drop down box.

Logical operations like AND/OR can be performed by selecting from the drop down box. Searches using incomplete information can also be done using one of the following options “approximately matches”, “exactly matches”, “is greater than”, and “is lesser than”. The complete query will look like an ordinary English sentence.

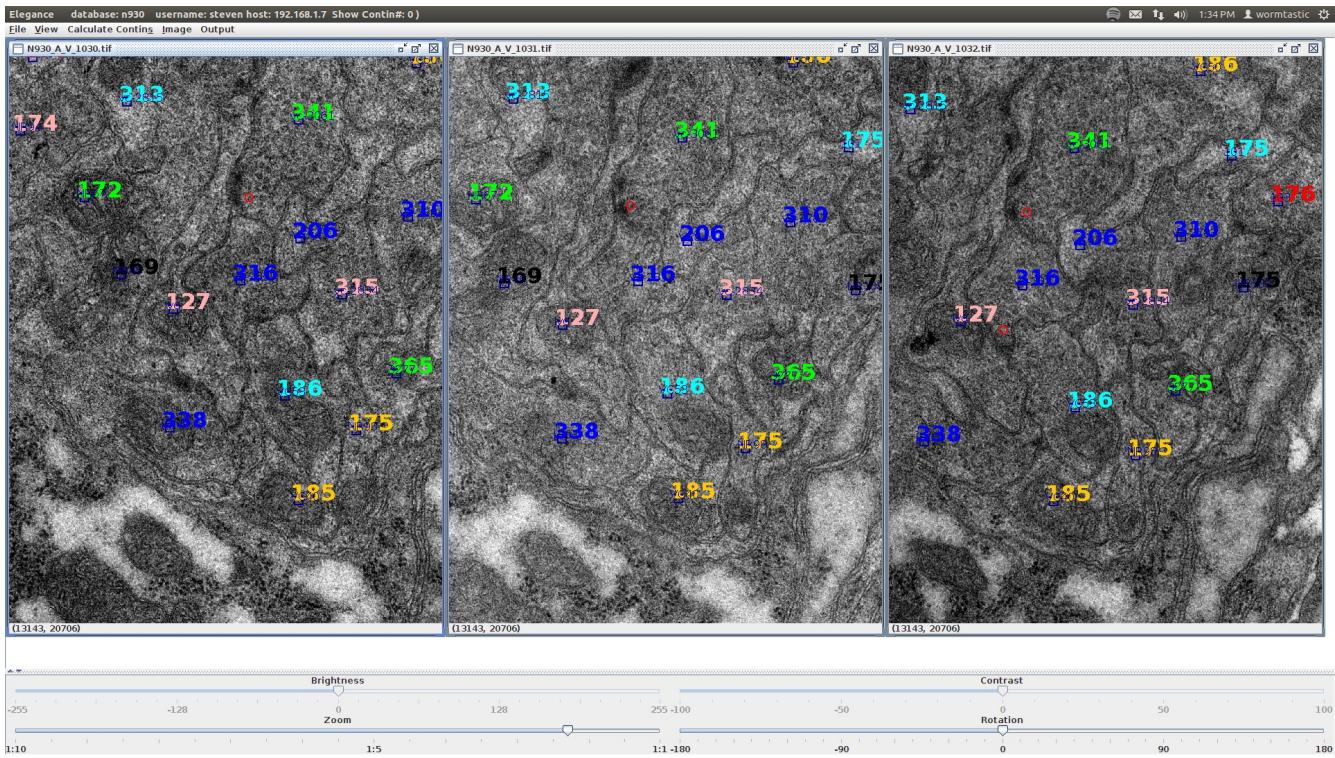
After creating the query press the "OK" button. The results if any will be displayed in a table. Multiple images can be selected by 'click' + 'ctrl/click' and/or 'shift/click' and can be loaded into the main window by clicking the "Load" button. The window can be closed by clicking the 'close' button or by exiting the window. The data in this search window will NOT be lost and can be viewed when the window is opened later. For a new search press the "back" button and reformulate the search query. The search window can be resized per the requirement. A successful search is shown below.



2.3 Working with Images

The images loaded from the load window are loaded into RAM and displayed in the main window as three separate smaller windows inside the main window. These smaller windows can be repositioned to wherever required by dragging the header and resized to whatever size required, by dragging the mouse from the side of the window. The header of the window of the current image (image window in focus) is drawn in purple color. The image number of the image opened is provided as the title for the internal window.

The user can translate the image by dragging the mouse on the image. The properties of the image like zoom, rotation, contrast, brightness can be changed by using the slider bars at the lower portion of the main window. These slider bars can be hidden/ unhidden by clicking on the downward arrow on the divider. The slider bars can be reset to their respective default values by choosing the “Image” -> “Reset Sliders” option on the main menu.



2.4 Locking and Aligning Images

The images can be locked by selecting "Image" → "Lock Images" from the main menu (or pressing 'Ctrl+L'). The locked images can now be translated together by translating any of the images.

Similarly the rotation or zoom can be changed by dragging the sliders. Contrast and Brightness cannot be changed in the Locked mode.

The images can be aligned by pixel coordinate (X,Y) by selecting "Image" → "Align" from the main menu (or pressing 'Ctrl+A'). Note that the images will be aligned such that the upper left hand corner of the image corresponds to the coordinate (0,0). All loaded images will be aligned.

2.5 Closing an Image

Any image can be closed by closing its corresponding window. To preserve the original image, the image properties like zoom etc. are stored in the database as attributes when the image is closed and are retrieved when an image is opened. As a result the original image is left untouched, but for preserving the previous state of the image-view quality, the rotation, brightness, contrast and zoom are re-imposed to the image on re-opening.

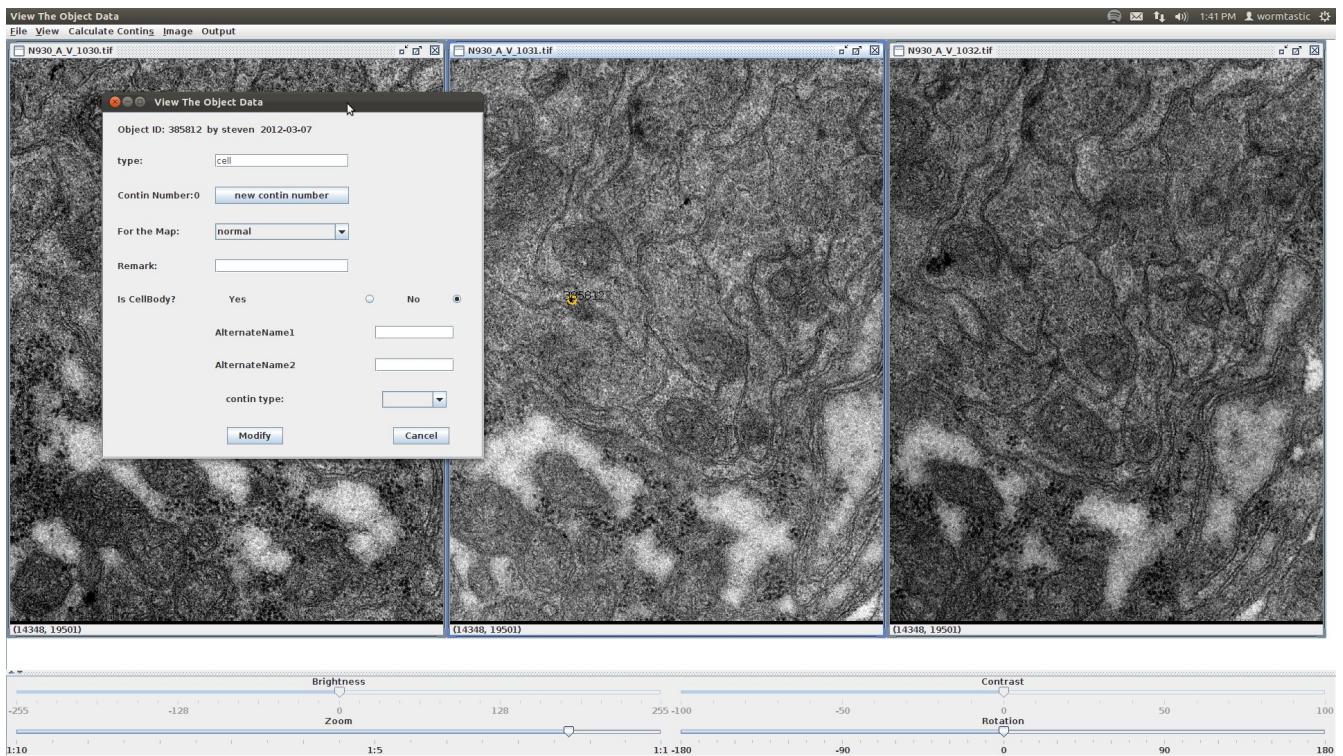
2.6 Entering Cell Objects

An object can be entered by double left clicking on any location on the image. The object will be displayed

by an open blue square. The object will automatically be entered in the MySQL database object table.

2.7 Selecting Objects

Alt + right click on an object to 'View The Object Data'. Its color will change to show that it has been selected. If the location of the object is to be changed, the object can be dragged after holding the mouse button pressed on the object. When the dragging is finished, the object will be at the new location. The object location is automatically modified in the application memory too. For deleting an object, select the object (the object should turn orange) and then press the "Delete" key on the keyboard. The object will be deleted from the database.



From this dialog box one is able to create a new contin number, designate whether the object is placed on a cell body, provide an AlternateName for the Contin, or change contin type.

To extend a neuron profile click on 'new contin number.' You may then extend the contin by single right clicking the corresponding neuron profile on the adjacent image. Adjacent images can be viewed by scrolling left or right with the arrow keys.

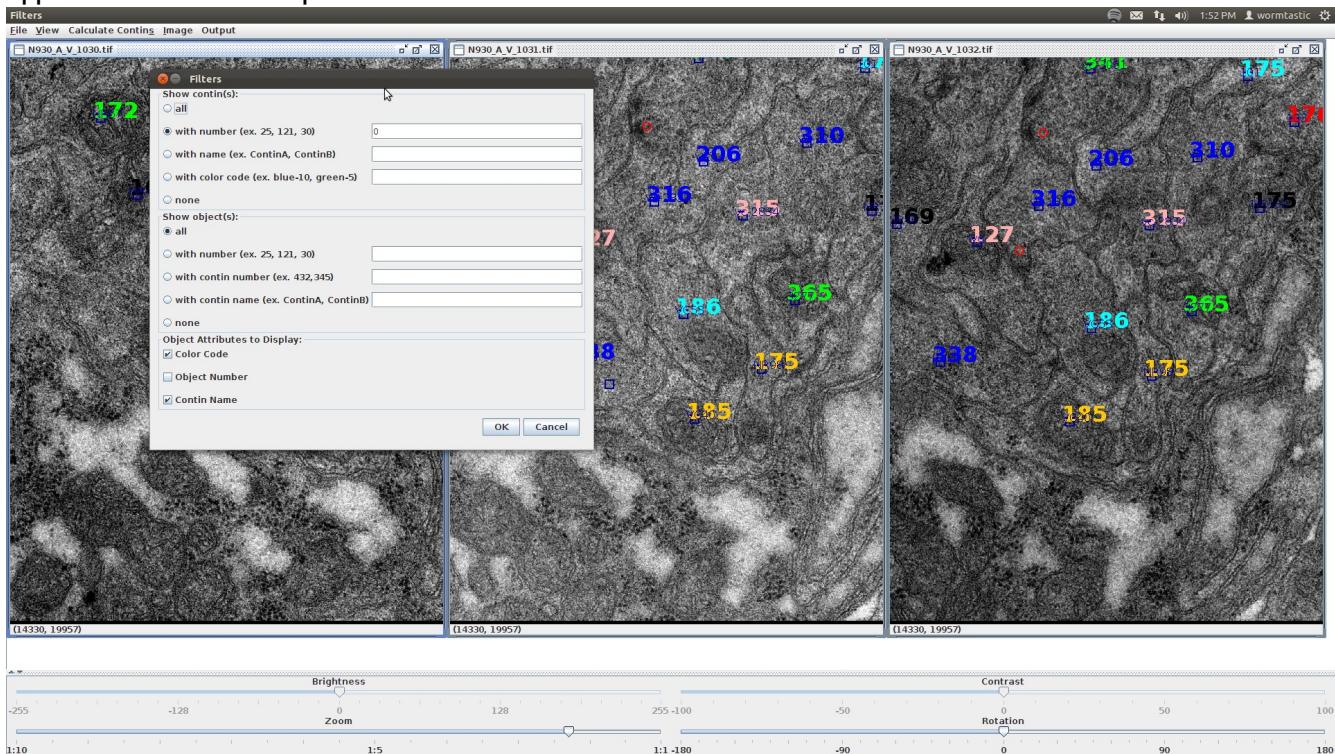
2.8 Objects Window

A list of the objects on the current image can be viewed in the form of a table by selecting the "View" -> "Objects Window" from the main menu (or by pressing 'Ctrl+P'). In addition to viewing, an object can be deleted from the database by selecting the relevant object and pressing the "delete" key.

The objects will be permanently deleted from the database. When the 'Objects Window' is open, if the user adds and/or deletes the objects on the related image, the information on the window can be refreshed by pressing the 'Refresh' button.

2.9 Object Display Options

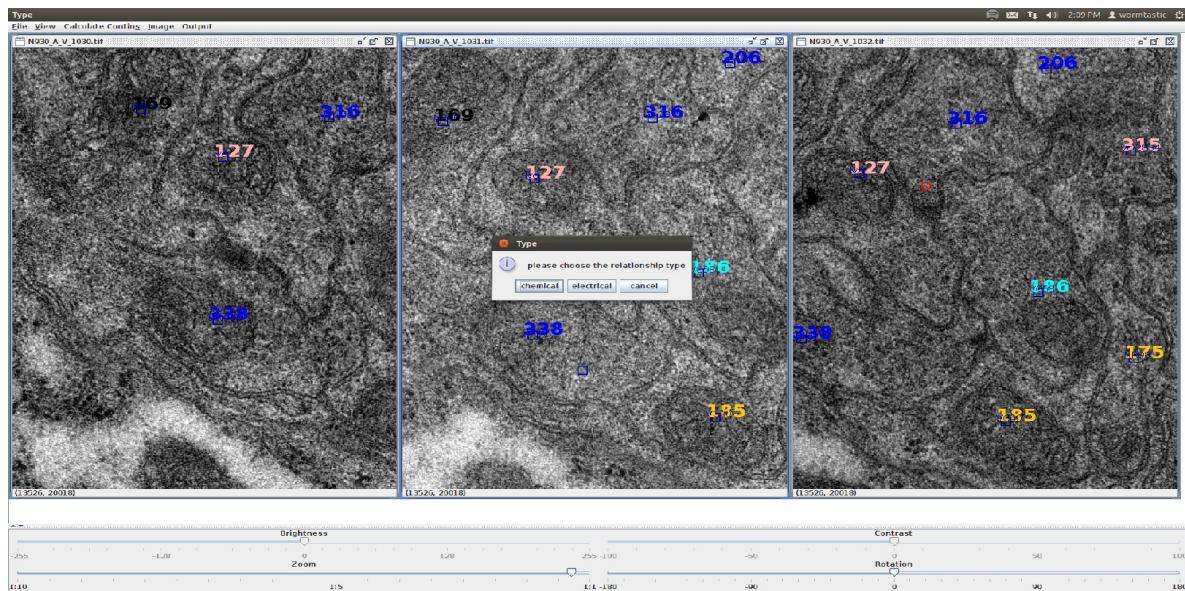
The user can toggle between "to view all objects" and "not to view any of the objects" by pressing 'spacebar'. However, it is NOT possible to selectively hide some of the objects. When all the objects are hidden, relationships if any will also get hidden. But when 'spacebar' is pressed again (to view all the objects), all the objects will appear but the relationships will still remain hidden.



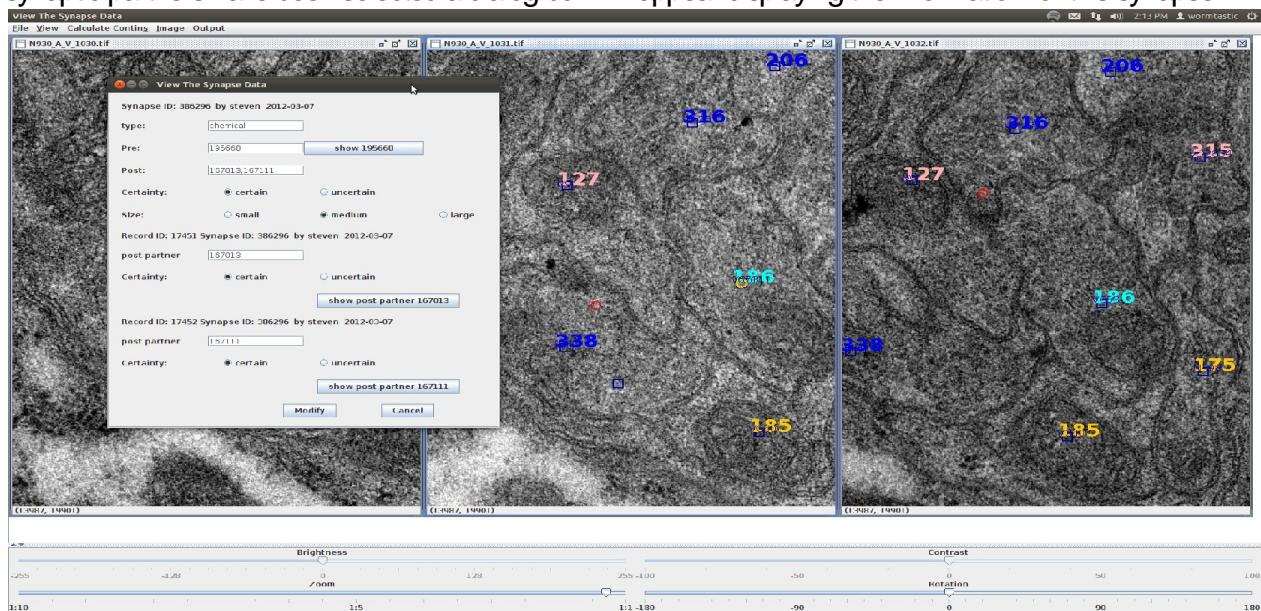
By 'Image' → 'Display Options' or pressing 'ctrl+d' a Filters dialog box will appear. From this menu one can select to view a specific subset of objects or contins. Additionally, objects can be displayed by color codes, objects numbers, and contin Names.

2.10 Entering Synapse Objects

To enter a synapse object between two or more cell objects hit alt + double left click. This will bring up a dialog box to choose whether to enter a chemical synapse or gap junction.



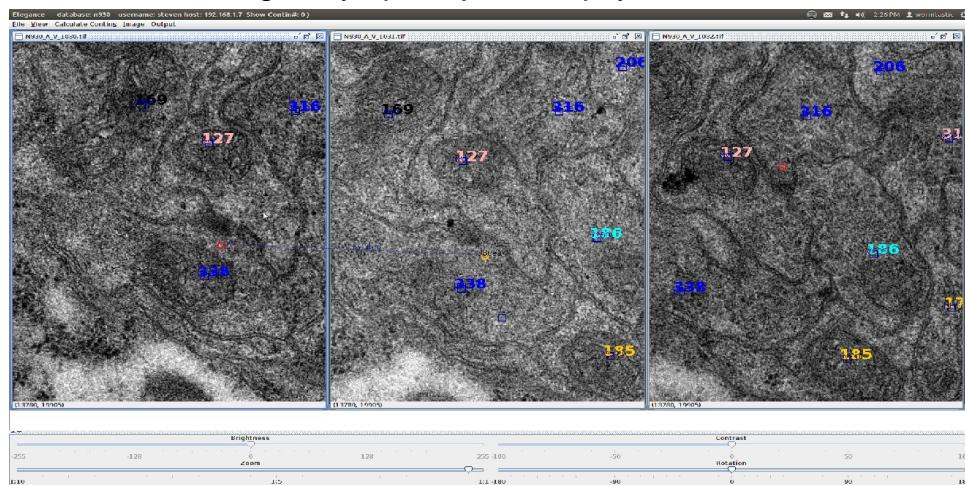
While still hold down the 'alt' key, first select the neuron profile containing the presynaptic density. After selecting the presynaptic cell, in a clockwise manner select each post synaptic partner with a single left mouse click. After all synaptic partners have been selected a dialog box will appear displaying the information for this synapse.



Assuming your entered information is correct, you have then hit enter or select modify to enter the object into the MySQL database. Gap junctions are entered in the same manner as chemical synapses, but instead selected 'electrical' as the Type after 'alt' + double left clicking the area of the image corresponding to the gap junction observed.

2.11 Connecting Contins

Synapse objects CAN NOT be extending by a single right click the way that neuron contins can be. To connect two corresponding synapse objects on adjacent images first 'ctrl+left click' the first synapse object, and then the second object. An infinite number of objects can be combined this way and will all subsequent be considered one contin. NOTE: Be careful when connecting objects as contins. A dialog box will appear to confirm that the objects you are connecting are indeed of the same contin. Contins are drawn as lines connecting two objects by Elegance. A contin connecting two synapse objects is displayed below.



2.12 Data Management Functions

Several procedures need to be followed after entering data to ensure a correct and proper representation of connectivity and cell maps.

Calculate cell contins: Calculating a cell contin begins from one object and finds those other objects related to the object by contin number. This information is stored in the 'relationship' table of the MySQL database. If the # of partners is >1, its type is then marked as a 'branch point.' The calculate function will also mark terminal objects as branch points. To calculate a cell object hold down 'c' and then left click on a cell object. Contins can also be calculated from choosing 'Calculate contins' from the main menu. Options are available to calculate by cell object # or contin #.

Update synapse contins: To ensure proper connectivity and generation of the 'synapsecombined' MySQL table select 'Calculate contins' → 'Update Synapse Contins' from the main menu bar. This calculation will typically take a few moments to complete.

Update Synapse Lists: To complete synapse and partner lists select 'Output' → 'Update Synapse Lists' from the main menu.

3. Generating Outputs

By following the steps in part 2 correctly, all of the MySQL tables will be populated.

3.1 Synapse and Partner Lists

Partner or Synapse lists can be generated by selecting 'Output' → 'Generate Synapse List' or 'Generate Partner List'. Both of these lists are displayed via a dialog box. The partner list will combine synapses while the Synapse list will individually list synapses. Both of these outputs can be saved as .csv files for subsequent analysis.

type	presynaptical	postsynaptical	synapses#	sections#
electrical	AVAL	RIMR	2	7
electrical	AVAL	URYDL	1	2
electrical	AVAL	URYVR	1	2
electrical	AVAL	PVCL	1	1
electrical	AVAL	PVPL	1	1
electrical	AVAL	ALA	1	1
chemical	AVAL	RIML	2	3
chemical	AVAL	AVDR	1	1
chemical	SAAVL	AVAL	24	56
chemical	SAADL	AVAL	7	17
chemical	ASHLR	AVAL	12	15
chemical	ASHLR	AVAL	7	15
chemical	RICL	AVAL	9	14
chemical	AUAL	AVAL	7	11
chemical	ADLL	AVAL	6	9
chemical	RIMR	AVAL	8	9
chemical	SDQR	AVAL	3	7
chemical	AIUNR	AVAL	2	6
chemical	RIML	AVAL	2	5
chemical	RIBL	AVAL	4	5
chemical	ABIL	AVAL	2	4
chemical	PVAL	AVAL	2	4
chemical	DVAL	AVAL	2	4
chemical	BAGR	AVAL	2	4
chemical	AQR	AVAL	4	4
chemical	ADLL	AVAL	2	3
chemical	PVCR	AVAL	1	3
chemical	RMGL	AVAL	3	3
chemical	URYVR	AVAL	1	2
chemical	AVR	AVAL	1	2
chemical	PAUL	AVAL	2	2
chemical	SAADR	AVAL	1	1
chemical	SIADR	AVAL	1	1
chemical	TCFHWRI	AVAL	1	1

type	presynaptical	postsynaptical	sections	syn id	image#
electrical	AVAL	RIMR	5	62029	N2UNR167
electrical	AVAL	URYVR	2	39969	N2UNR118
electrical	AVAL	RIMR	2	62044	N2UNR050
electrical	AVAL	URYDL	2	62283	N2UNR078
electrical	AVAL	PVPL	1	61763	N2UNR114
electrical	AVAL	PVCL	1	63913	N2UNR102
chemical	AVAL	RIML	2	62205	N2UNR116
chemical	AVAL	AVDR	1	62201	N2UNR123
chemical	AVAL	RIAF	1	62229	N2UNR115
chemical	SDQR	AVAL AIBL	5	3278	N2UNR176
chemical	SAAVL	SMDR,AVAL	5	14827	N2UNR134
chemical	SAADL	AVAL,SMDR	5	15077	N2UNR018
chemical	AIUNR	IIZDRI,RIML,AVAL	5	62297	N2UNR056
chemical	SAAVL	RIMR,AVAL	4	9531	N2UNR161
chemical	SAAVL	RIMR,RIML,AVAL	4	15074	N2UNR122
chemical	RICR	AVAL,SMDR	4	28045	N2UNR099
chemical	SAAVL	AVAL,RIAF	4	39114	N2UNR069
chemical	ADLL	AVBL,AVAL	4	56354	N2UNR116
chemical	SAADL	RIML,AVAL,RIMR	4	60182	N2UNR058
chemical	RIML	AVAL,SAAVL	4	62407	N2UNR070
chemical	AIUB	SMDR,AVBL,AVAL	3	6135	N2UNR167
chemical	AIUC	AVAL,SMDR	3	14920	N2UNR040
chemical	SAAVL	SMDR,AVAL	3	14921	N2UNR140
chemical	SAAVL	AVAL,RIMR,RMFR	3	60005	N2UNR115
chemical	RICR	AVAL,SMDR	3	39100	N2UNR094
chemical	SAADL	AVBL,RIMR	3	39144	N2UNR063
chemical	SAADL	AVBL,RIMB	3	60170	N2UNR044
chemical	SAADL	AVAL,RIMR	3	60184	N2UNR055
chemical	SAADL	RIML,AVAL	3	60187	N2UNR054
chemical	ASHLR	AVBL,AVAL	3	60890	N2UNR091
chemical	ASHLR	AVBL,AVBL,BAGL	3	61766	N2UNR067
chemical	AUAL	AVBL,RIAL,RIBL	3	61804	N2UNR118
chemical	PVCR	AVAL,AVBL	3	62204	N2UNR118
chemical	SAAVL	RIMR,AVBL	2	6522	N2UNR159

3.2 Adjacency Matrices

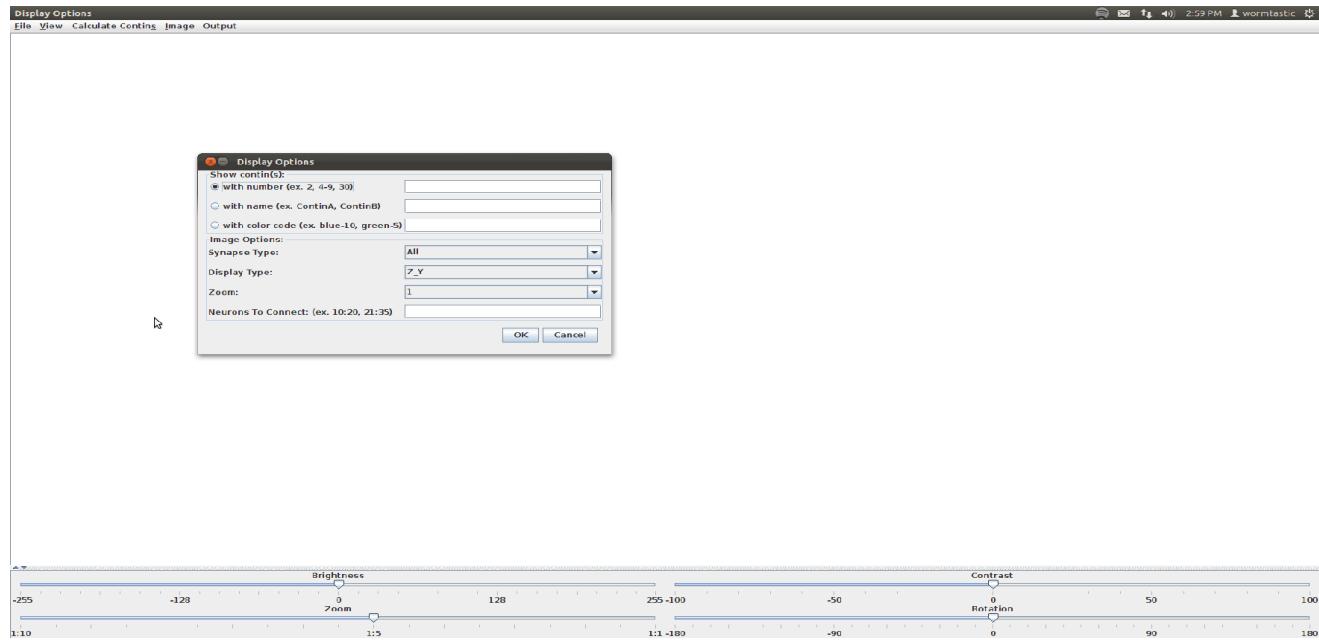
Chemical or Electrical adjacency matrices can be generated by selecting 'Output' → 'Chemical Adjacency Matrix' or 'Electrical Adjacency Matrix'. One then has the ability to select which subset of neurons to include in the matrix as well as whether to subset by connection weight. These matrices will take several moments to generate and are saved in the Elegance home directory as .csv files.

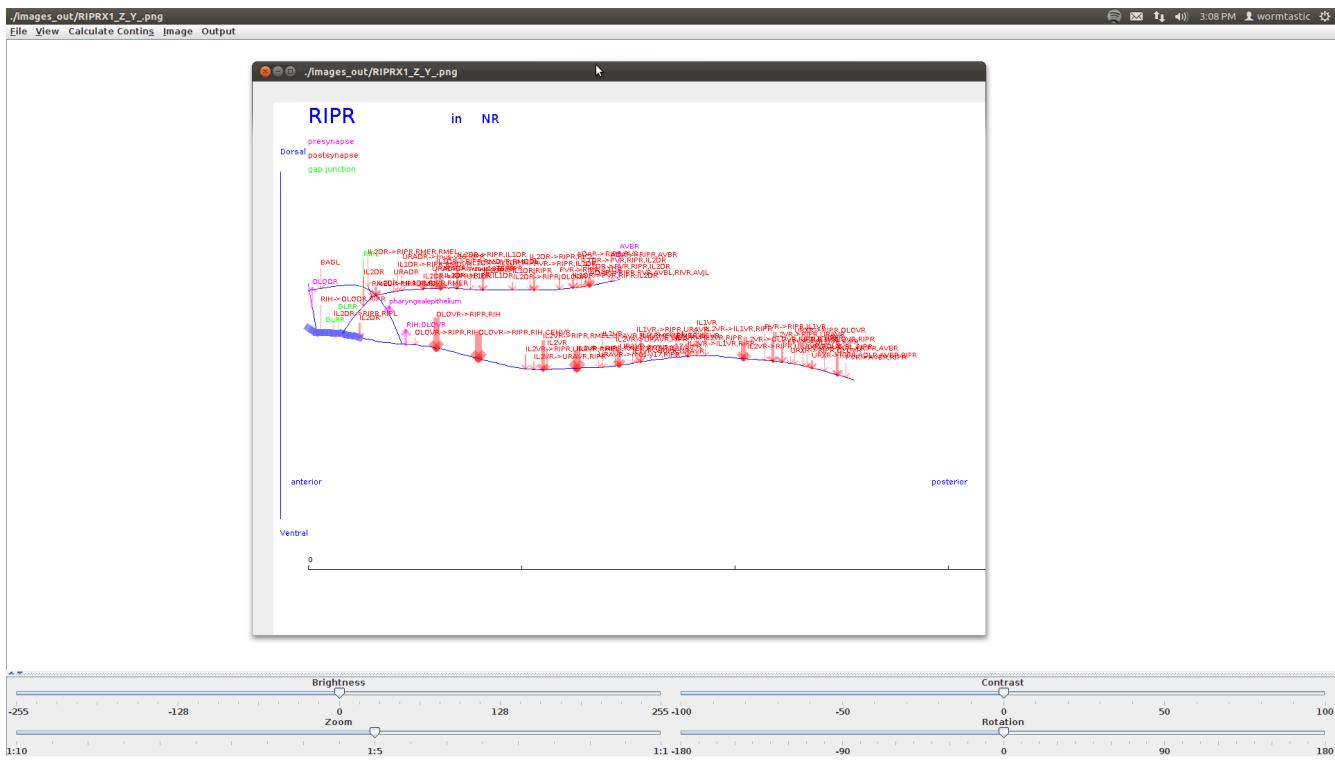
3.3 2D Output

To show 2D display options select 'Output' → '2D Display' or hit 'ctrl+2'.

From this dialog box you have the option to display single or multiple neurons by either contin number, alternate name, or their color code. Additional image options include synapse inclusion (yes/no) and whether to display only pre/post synaptic. The Default view is Z_Y but Z_X is also available along with various zoom factors.

Another option exists to display the synaptic interaction between two neurons. To accomplish this enter two neuron contin #'s in the Neurons to Connect box.

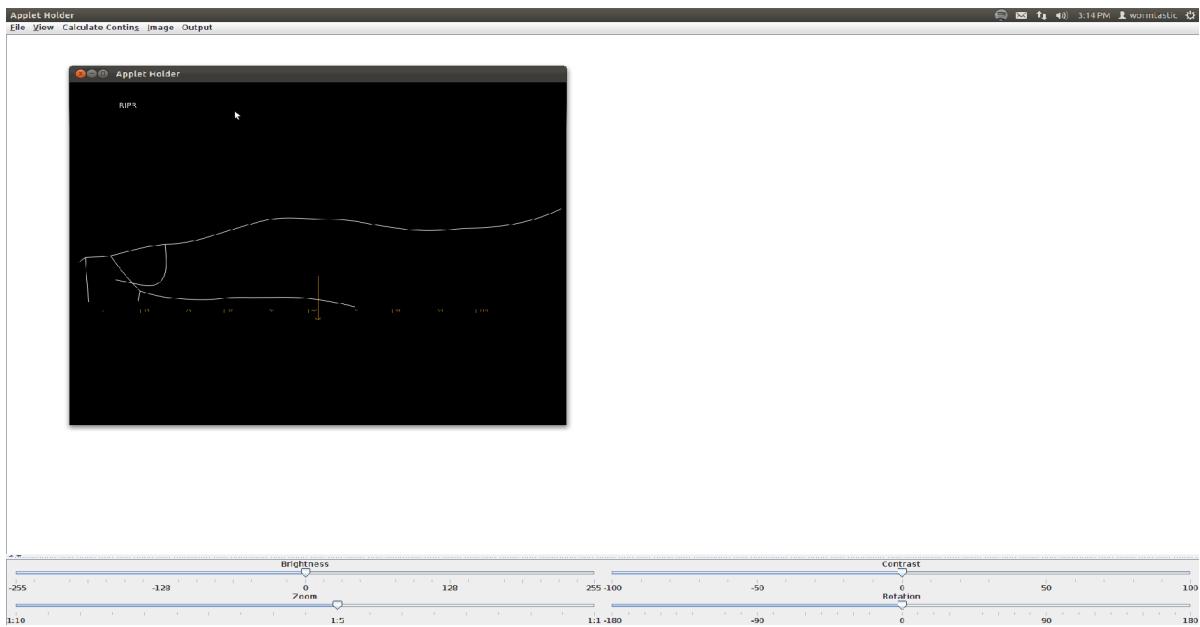




A representative neuron map is shown above. Images are displayed as a separate window but are also saved as .png files in the 'image_out' folder of the Elegance home directory.

3.4 3D Output

To view a skeleton Diagram of a neuron in 3D select 'Output' – '3D Display'. One is then prompted to enter a contin alternate name. A rotating model of the neuron skeleton will be displayed in a separate window.



4. Shortcuts and Tips

Keystroke	Function
Left Click	Scroll Across Image
Right Click	Extend Contin
Double Left Click	Add New Object
F5	Refresh
Spacebar	Hide / Show All Objects
Alt + Double Left Click	Add New Synapse Object
Alt + Left Click	Modify Object
Alt + E	Enter Image
Alt + F	File
Alt + I	Image
Alt + S	Calculate Contins
Alt + V	View
Ctrl + Left Click	Connect Two Objects
Ctrl + 2	2D Display
Ctrl + A	Align Images
Ctrl + D	Object Display Filters
Ctrl + F	Find Image
Ctrl + L	Lock Images
Ctrl + M	Modify an Image
Ctrl + N	Contins Window
Ctrl + P	All Objects
Ctrl + S	Select an Object
Ctrl + X	Exit

- All the extra windows in this application are non-modal which means that the user can change the focus between the open windows using ALT-TAB.
- The application is multithreaded and native code acceleration (using Java Advanced Imaging API -JAI) has been used in this software for faster image processing. As a result the extra open windows should not degrade the performance of the application.
- Database connections on Elegance startup are pooled and re-used to allow for smooth scrolling across images.
- All the images are stored in the RAM for faster image processing. We suggest using a minimum of 8GB of RAM. 16 or 32GB will increase the number of images able to be concomitantly loaded.
- The last ‘opened image’ is automatically made the ‘selected image’ .
- Elegance is platform independent. All necessary .jar files are included in the Elegance home directory. We currently run Elegance using Ubuntu 11.10. Unix-based operating systems are preferable.