The Neuron Navigator:

Exploring the Information Pathway through the Neural Maze

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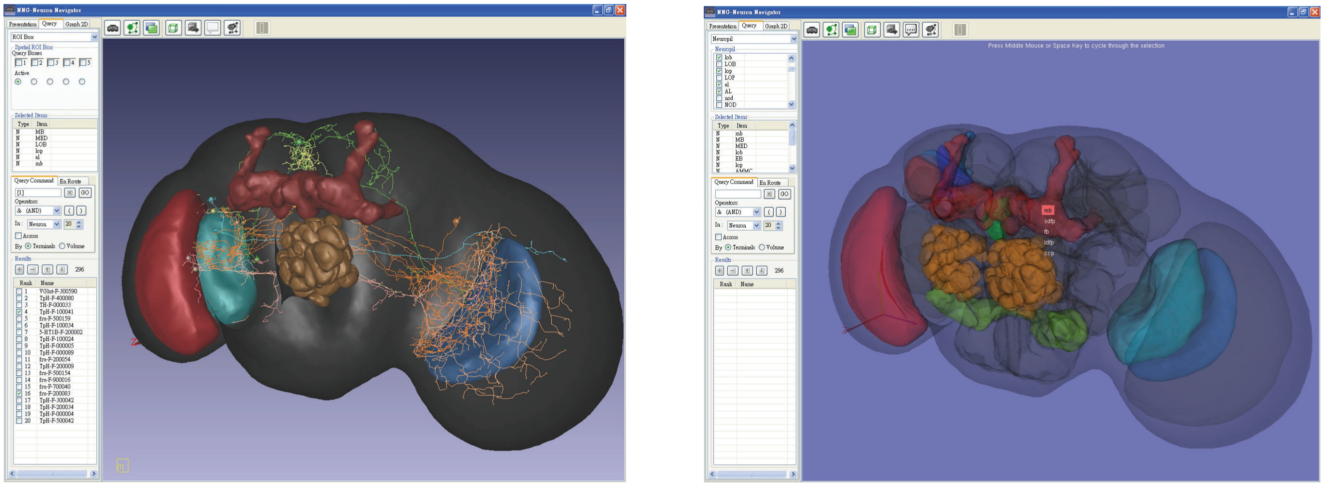

Type	Item
N	MB
N	MED
N	LOB
N	lop
N	al
N	mb

Query Command: [1]

	Rank	Name
☐	1	VGlut-F-300590
☐	2	TpH-F-400080
☐	3	TH-F-000033
☑	4	TpH-F-100041
☐	5	fru-F-500159
☐	6	TpH-F-100034
☐	7	5-HT1B-F-200002
☐	8	TpH-F-100024
☐	9	TpH-F-000005
☐	10	TpH-F-000089
☐	11	fru-F-200054
☐	12	TpH-F-200009
☐	13	fru-F-500154
☐	14	fru-F-900016
☐	15	fru-F-700040
☑	16	fru-F-200083
☐	17	TpH-F-300042
☐	18	TpH-F-200034
☐	19	TpH-F-000004
☐	20	TpH-F-500042

Neuropil:
☑ lob
☐ LOB
☑ lop
☐ LOP
☑ al
☑ AL
☐ nod
☐ NOD

Type	Item
N	mb
N	MB
N	MED
N	lob
N	EB
N	lop
N	AMMC

(a)

(b)

Fig. 1. (a) NNG draws neurons using tracing lines

(b) Neuropils can be drawn transparently.

ABSTRACT

Recent advances in microscopic imaging technology have enabled neuroscientists to obtain unprecedentedly clear images of neurons. To extract additional knowledge from the tangled neurons, for example, their connective relationships, is key to understanding how information is processed and transmitted within the brain.

In this paper, we will introduce our recent endeavor, the Neuron Navigator (NNG), which integrates a 3D neuron image database into an easy-to-use visual interface. Via a flexible and user-friendly interface, NNG is designed to help researchers analyze and observe the connectivity within the neural maze and discover possible pathways. With NNG's 3D neuron image database, researchers can perform volumetric searches using the location of neural terminals, or the occupation of neuron volumes within the 3D brain space. Also, the presence of the neurons under a combination of spatial restrictions can be shown as well. NNG is a result of a multi-discipline collaboration between neuroscientists and computer scientists, and NNG has now been implemented on a coordinated brain space, that being, the *Drosophila* (fruit fly) brain. NNG is accessible through: http://211.73.64.34/NNG.

KEYWORDS: neuroscience, 3D image database, neuro-image visualization, connectivity search

1 INTRODUCTION

The functions of the brain reside on the network of neurons within the skull. Imaging of single neurons in three dimensional space is now possible due to recent advances in microscopic imaging technology. Neuroscientists are now able to acquire clear images of the complicated neuron structures that were previously unobtainable. However, the ability to process the images and extract meaningful information from the tremendous amount of data posts another great challenge for the modern neuroscientist.

Studying the systemic neuroanatomy of the brain's architecture is the foundation for understanding how the brain works and how the information is processed and transmitted within. The brain of the *Drosophila melanogaster* (fruit fly) is a convenient model for studying brain structure/function relationships because it is relatively small in size as compared to the human brain (which contains many million more neurons and, as such, is too large and complicated to study) yet it is still capable of sophisticated behavioural expression. Also, the *Drosophila*'s genetic background has been thoroughly studied and its brain has strong similarities to mammalian brains [1].

Whenever possible, researchers have used 3D visualization tools to assist them in analyzing complicated data. It has recently been shown that a systemic collection of 3D images for single neurons within the Drosophila brain is feasible. Also, a unified coordinate system for the locations of these neurons has been constructed as part of this project. To further produce meaningful results, a modular 3D image database has also been constructed in

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a standardized brain space of *Drosophila* [2]. The primary task now is how to manage these tens of thousands of neurons so researchers can select an ensemble of neurons and reveal their functional significance. Until now, knowing how the neural signals are transmitted within the neural network and through what neurons they travel were questions that could not be answered because the proper tools to mine such large quantities of data did not exist.

In a unified and coordinated space, the spatial relationships among the neurons can be easily queried and searched if a proper database has been constructed. We have processed more than 10,000 individual neuron (from [2]) to construct a 3D neuron image database. The database stores the volumetric and tracing line information, as well as the positions of terminals and somas of neurons.

It is impossible to understand the neural network without knowing the details of the connections between the objects. For this project, we developed a novel system called the Neuron-Navigator (NNG) to help biologists and neuroscientists analyze and process neuron data. The cross-platform NNG was built up with a visual interface and integrated with the above mentioned database.

The visual interface allows users to visualize and interact with the data (Fig. 2). Through the interface, users may query the whole coordinated brain space to find out relationships among neurons and brain parts. A tolerance region has been set to determine the connectivity among the neurons. Using this specially designed interface, researchers can even look for neurons that pass through a single or multiple Regions Of Interest (ROI) or relay neural connections through terminals of neurons. NNG's query interface accepts text and graphic queries and the results are shown as networks of neural skeletons within the 3D brain space.

2 MANAGING NEURON IMAGES

There are currently several software packages available, such as Amira [3] and ImageJ [4], that are able to process neuron images. However, these packages are mainly used to classify and process image data for visualization. They are good visual analysis tools but they are not designed to help researchers explore the patterns of neuron networks hidden within the images.

An image database or a digital atlas may facilitate neuroscientists to study the complicated neural structure/function relationships. The first fly brain database was first reported in 1995 [5], although rather primitive at that time. The demand for use of image databases to analyze neuron populations is increasing [6-10]. Recent advances in computer hardware and software have made this task feasible. As an example, the small family of neurons in C. elegans has already been completely categorized, although not in a physiologically relevant 3D space as of yet [11].

Research regarding the use of neuron image databases to find the connective relationships between neurons is rare. XANAT is an early program that allows users to graphically query anatomical connections within the brain. With XANAT, users can find connectivity information by specifying the brain Region Of Interest (ROI) [6]. However, the images that XANAT uses are from the subdivision of the brain rather than at the neural level. BrainGazer recently introduced more advanced query techniques with their software which allows users to sketch arbitrary paths and immediately obtain nearby objects [12]. Section 6 contains a detailed discussion between NNG & BrainGazer.

An ideal brain atlas should incorporate several basic characteristics such as the ability to accurately represent details in 3D, a powerful interface for searching and visualizing data, and broadband internet access [8]. Once a reliable neural brain network is established, the functional module can be hypothesized according to the results from other studies including behavioral and physiological methods [13].

The Spatial Database Management System (SDBMS) can be used to manage and process image data of normalized and unified neuron image data sets. SDBMS is a database management system that includes additional capabilities for handling spatial data. It also offers spatial data types (SDTs) in its data model and query language [14].

The main application area driving research in SDBMS is Geographic Information System (GIS). GIS can be used to compute the shortest path from one node to another, as an example, when it is used to analyze the connectivity between two cities. GIS has also been applied to 2D-3D hybrid building models, and indoor routing in the buildings can be performed using other applications [15].

SDBMS can be applied to many different domains besides GIS. For example, in the medical domain, doctors can use it to recognize similar conditions in different patients [16] [17]. Molecular biologists use SDBMS to compare the topologies of different genomes through a system which supports semantic and analytical queries and image content mining [18]. Also, SDBMS can be used to issue queries such as "Display the locations of all data registered to the cerebellum of a rat" or "Retrieve data based on the location by selecting a brain region" [19]. Also, astronomers use SDBMS to interactively explore data in the system, searching and analyzing the stars among the galaxies [20]. As long as objects can be situated in a unified space, SDBMS can be used to help researchers better understand the information.

3 SYSTEM DESIGN

The main objective of the NNG is to uncover the knowledge hidden within the scattered neurons. With an unprecedented number neuron images data sets on hand (10,000, and counting), the challenge is how to best organize the data and provide researchers a friendly environment in which to observe and analyze the data. The tools provided in NNG, which include visualization, interaction and a query system, ease the user's work

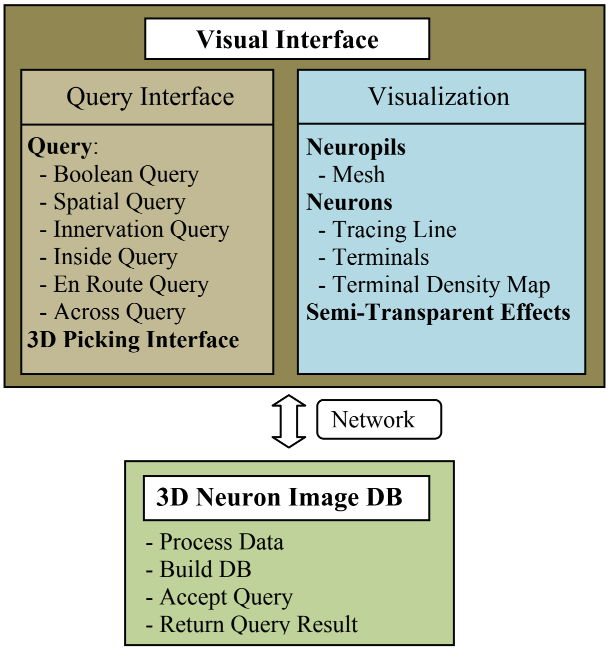

Fig. 2. NNG architecture overview

while finding neuron connectivity and innervation patterns.

Fig. 2 shows an overview of the architecture of NNG which consists of two main modules: (i) the visual interface at client site, and (ii) the backbone image database. The client site visual interface is built on two components--the query interface and the visualization interface. The query interface handles the query commands from the user. The query results can be shown in a list table or directly in the scene. Here, data are presented in different ways and allows direct interaction with the data. The 3D neuron image database stores the spatial data and other information regarding the neurons and neuropils. The 3D neuron image database accepts the query commands and returns the results. The database does not need to reside on the same system as the interface - via a network, it is possible to access the database remotely.

Categorized neurons were generated by engineering the green fluorescent protein (GFP), under the control of a specific gene into the *Drosophila* embryos. Through a specific gene promoter, the expression of the GFP protein is associated with several genetic features (e.g. for different neurotransmitters or sexual dimorphic genes). By observing the presence of the GFP protein and the expression of the gene, the function of the neurons is illustrated. The GFP images were acquired using confocal microscopy. The category under which the neurons fall is determined by the production of the gene it is involved in. MARCM [21] is used to reveal not only single neurons, but also their birth time during developmental stages.

3.1 3D Neuron Image Database

A simple way to query the correlation between elements taken from the volumetric data of neuron images is to use a database to store the spatial information, this is crucial for the functionalities described below. Although the neurons are rendered using tracing lines, the neuron spatial information stored in the database includes not only terminals, but also voxels of neuron images data. The *Drosophila* brain contains up to 130,000 neurons, thus, the total size of the volumes representing the neurons is very large. The question then becomes how to most efficiently store and retrieve the data in the database.

All of the neuron images stored in the NNG's 3D Neuron Image Database have been pre-processed and normalized. The images are put into unified and standardized *Drosophila* brain space; therefore, SDBMS is suitable for this case. Several types of data were acquired after pre-processing, including the original volume data, neural information (e.g. the normalized coordinates, neuron names, etc.), and the tracing lines. The 3D Neuron Image Database stores all the information to create a hybrid 3D image database. It is composed of two main tables--the Neuro-information table and the 3D Neuro-space table. The Neuron-information table contains indices of the neurons, normalized coordinates, filenames, and the other information. The 3D Neuro-space table contains both the 3D coordinates of the occupied voxel of the neurons and the terminals of the tracing lines. Neurons are categorized according to the possible neuro-transmitters (Cha, Gad, vGlut, Trh, TH, and Tdc2) used and some characteristic genes (e.g. sexual dimorphic *Fru*).

In order to create the 3D Neuron Image Database, a unit voxel in the standardized fruit-fly brain space is predefined according to a tolerance region that is set by the user to determine whether a connection appears or not. In our setting, the dimensions of a unit voxel are 2 x 2 x 2 pixels, and the dimensions of standardized brain space are 1944 x 1222 x 740 pixels. The standardized brain space is divided by the unit voxel (see Fig 3(a), but the grids in Fig. 3(a) do not represents the real size of a unit voxel). Then every neuron's image data is processed to find the corresponding occupied voxels in the standardized brain space. A new record is inserted into the table if any of the occupied voxels are not in the table. The record includes the coordinate of the unit voxel and the index of the neuron. If the unit voxel already exists in the table, then the index of the neuron that corresponds to the record is added. The locations of the terminals of the tracing lines are also recorded into the table (See Fig 3). Finally, the normalized coordinates and other information, such as the file location, are saved in a different table (See the Neuro-Info table in Fig. 3). These tables are generated using a MySQL database. The MySQL database can be easily accessed through network, so the database can reside in remote machine.

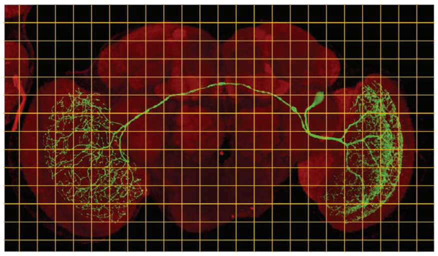

Fig. 3 (a)
Brain space is divided by unit voxel, the grids are not at the actual size of the unit voxel, just conceptual.

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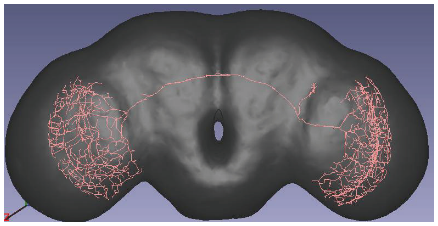

Fig. 3 (b)
Corresponding tracing line of Fig 3(a)

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Neuron Information

⇩

Neuro-Info table

Index	Type	Bounding box Coordinate..	Volume File	Lineset File	Terminal Amount
1	2	0 0 0 5 6 7	/…	/…	10
2	4	0 0 0 3 9 5	/…	/…	15
3	…	…	…	…	…

Neuro-Space table

X	Y	Z	Volume Index	Terminal Index
0	0	0	1 2	1-4 2-3
0	0	1	1	1-5
0	0	2	2	2-4

Fig. 3. 3D Neuron Image Database

After the database is created, a friendly interface for the user to submit queries and observe query results is needed. While querying spatial connectivity, the user often has to choose a neuron of interest or a spatial region in the 3D display and then submit a query. We have designed the visual interface to help users perform such queries and observe the query result.

3.2 Visualization

3.2.1 Neuron and Neuropil Rendering

The stacked images acquired with confocal microscopy are volumetric by nature; volume visualization seeming to be the most popular way to render neuron image data [12] [22]. However, when rendering as little as tens of neurons volumetrically, even when the blending function is enabled to create a semi-transparent effect, the occlusion effect sometimes prevents the user from being able to view the intertwined neuron structure. With NNG, loading, unloading and displaying hundreds of different neuron image data sets from the query results at the same time is a common task. Frequently loading and unloading such a large amount of neuron volume images will dramatically reduce the system's performance.

For the purpose of real-time interaction and display, the neuron images are pre-processed to generate tracing lines. The technique used in this paper is described in [23]. The NNG renders the neurons in tracing lines. The tracing line color is chosen according to the neuron-transmitter category which the neuron belongs to. Another advantage of using tracing lines in the way is that the user is able to identify the terminals of the neurons which are located at the end of the branches of the tracing lines. NNG draws spheres to indicate the terminals. The somas are drawn as larger spheres (Fig. 1 (a)). With terminal location information, the user is able to query connecting neurons that pass through specific terminal areas.

To render the neuropils, the image data was pre-processed to create a triangular mesh representation. The number of triangles of each individual neuropil was scaled down to around 4,000. In situations where rear neuropils are hidden behind other neuropils, NNG utilizes a depth peeling algorithm [24] that allows the user to render neuropils semi-transparently (Fig. 1 (b)).

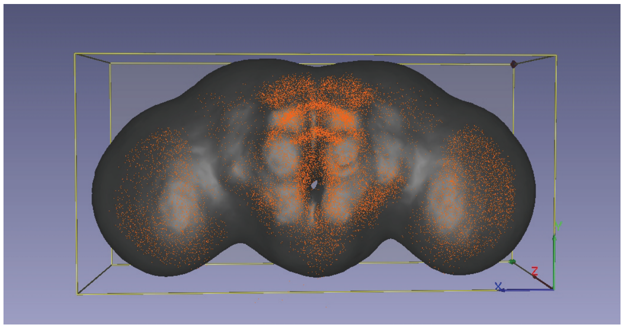


Fig. 4. Render of the terminals of dTdc2 in point clouds. The two central bands from the fan-shaped body are easily seen.

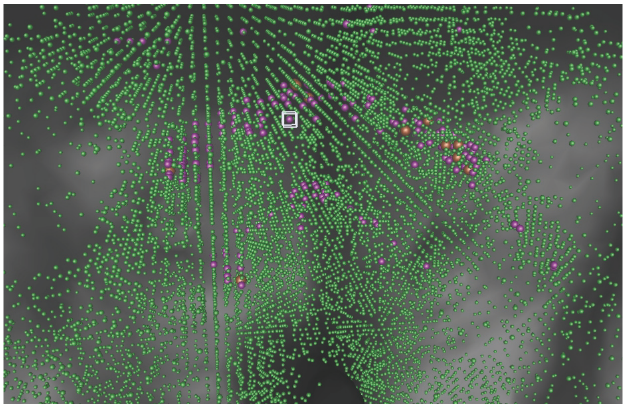


Fig. 5. Terminal Density Map. The sphere size represents the density at the location. Click the sphere to query.

3.2.2 Terminals and Terminal Density Map

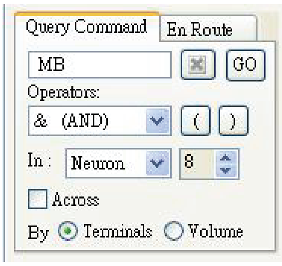
NNG incorporates a special and unique visualization tool, the terminal and density map of terminals, which renders all of the terminals in point clouds. Using the neuron tracing line information, NNG can render terminals whose distribution can generate pattern effects (e.g. see Fig. 4) that can be easily recognized. Researchers can use the terminal map to compare relationships between the terminals' spatial distributions and the shapes of the neuropils. In terminal map mode, a clipping plane or slab can be enabled to observe the terminal distribution in a particular slice. The terminals appear more and less concentrated in regions where the neuropils are located. This suggests that terminals determined by neural traces may be neurologically meaningful.

The terminal density map (Fig. 5) uses spheres of different sizes and colors to represent the spatial distribution of terminal density. If users are interested in finding the neurons that pass through high-density regions, they can use the mouse to pick that target sphere, and then send query to the database to obtain the result.

3.3 Query Interface

NNG's special capability is in finding correlations between neuron connectivity. By linking this functionality with the interface, we improve the usability and accessibility requested by neuroscientists.

3.3.1 Text Query Interface



MB

Operators: & (AND)

In: Neuron 8

☐ Across

By ● Terminals ○ Volume

Fig. 6. Query UI

The text query interface allows the user to input query commands. It can be a single object or combination query that uses binary operators to associate multiple objects together for more advanced queries. The object can be a neuron, a neuropil, or a spatial ROI box.

NNG provides five operators, **AND**, **OR**, **NOT**, **INSIDE**, and **INTERSECTION**. The capabilities of the query function are explained in the next section. Fig. 6 shows the text query interface. To input an object, the user simply types in the object's name using a keyboard or double-clicks the desired object on the screen and the name of that object automatically fills the text query field.

3.3.2 Graphics Interface

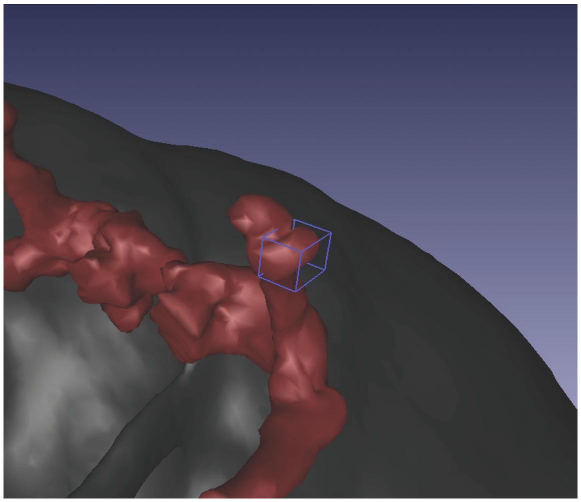
NNG provides several ways for the user to directly interact with the data. The user can use the mouse to perform basic operations such as rotation, translation, and zoom simply by clicking and dragging it. The user can also acquire the name of the neuron simply by moving the mouse cursor over it, as well as other information such as neuro-transmitter and gene information by clicking the neuron in annotation mode.

The user is able to use the mouse to adjust the position and shape of the ROI box. The ROI box can be elongated or shortened by dragging any of the three front-faced facets while in box editing mode. This is a very convenient way for the user to assign and adjust the box position.

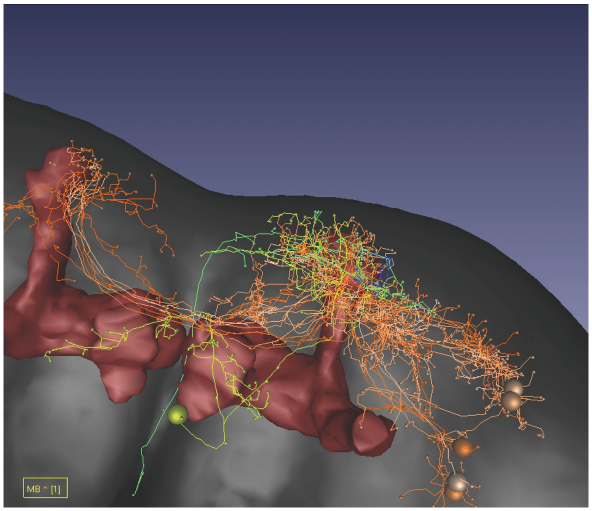
4 NEURON CONNECTIVITY QUERY

NNG incorporates various query techniques to help neuroscientists find hidden patterns within neuron images. The neurons in the query results can be displayed on the visualization interface to let users examine them and verify their results.

Users can also set the query option by *terminals* or by *volume* (see bottom of Fig. 6). The 3D neuron image database stores not only the image volume information, but also the terminals' location information acquired from the neurons' tracing lines. Query by terminals will only look for neurons which possess



7(a) Intersection query- The user adjusts the ROI box to enclose the upper region of the mushroom body and then submits query.



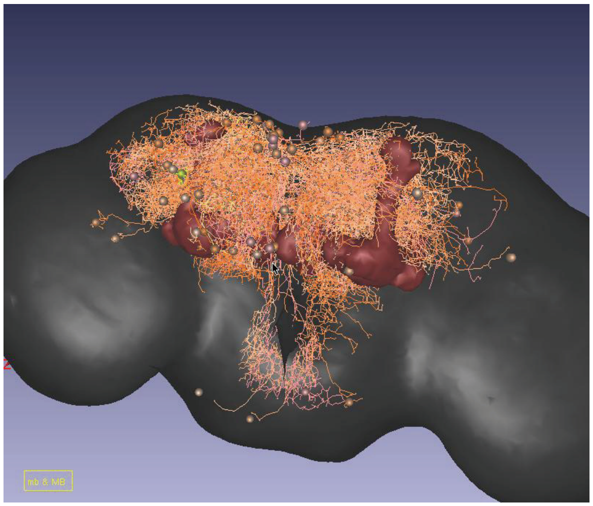
7(b) Only the neurons passing through the intersection are returned. This figure only shows part of the results. Green : *Fru*, Yellow: *Cha*, Orange: *vGlut*. *Fru*, *Cha*, and *vGlut* represent different genetic drivers.

Fig. 7. Intersection Query

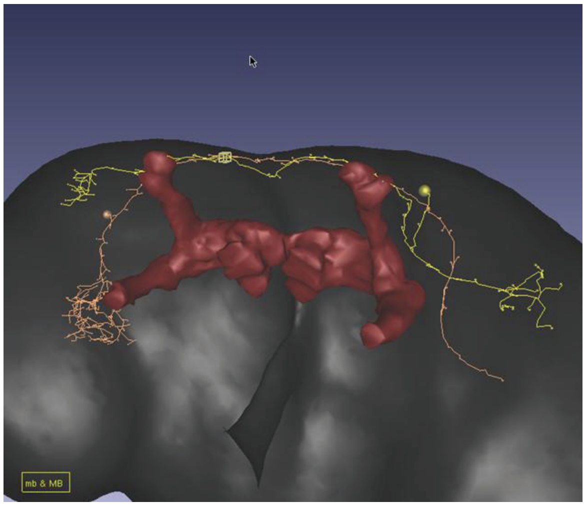
terminals located inside of the target object. On the other hand, query by volume will examine the voxels in the database to find the neurons which have voxels that meet the query criteria.

NNG's query technique design is quite unique. In this section, we will go into more detail regarding NNG's query functions' capabilities. In the following description, O_i represents an object in the scene which can be a neuron, a neuropil, or a ROI box.

a. ***Single Object Query***: The basic query is the single object query. It is used when the user is interested in finding what neurons or neuropils are connected to a specific object. The object can be a neuron, neuropil, or a ROI box. When the user performs a single object query, the database will return all the neurons or neuropils which have spatial intersections with the object. For example, "MB" is shown in the query command field in Fig. 6. When the user presses the "GO" button to send the query, the query results will show the neurons that have terminals located within MB (i.e. right-side mushroom body)



8(a) The query results of mb & MB. Too many scattered neurons make it difficult for the user to identify specific neurons.



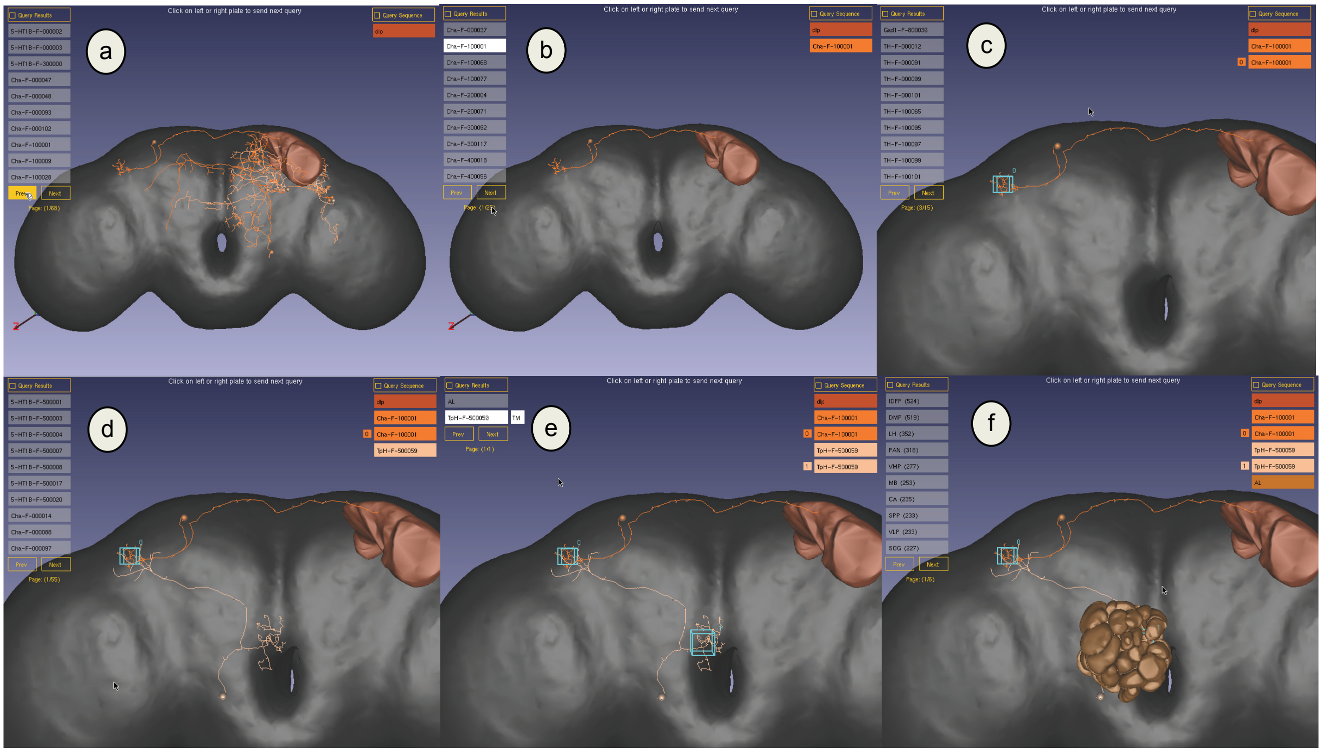
8(b) By enabling the across box, the user can find the neuron(s) passing through the specific region (from 8 (a)).

Fig. 8. Across Query

b. ***Boolean Query***: Using a Boolean operator, the user can combine multiple single object queries to perform more sophisticated queries. The following shows some examples:
   i. O_1 **&** O_2 :
      Find the neurons/neuropils passing through both O_1 and O_2, such as "**OG & og**"
   ii. **(**O_1 **AND** O_2 **) OR (**O_3 **AND** O_4**)**
      The union of two **AND** query results
   iii. **(**O_1 **AND** O_2**) NOT** O_3 :
      Find the neurons/neuropils passing through **(**O_1 **and** O_2**)** but not passing through O_3

c. ***"Inside" Query***: When the user wants to know which neurons or neuropils are located within a ROI, he can specify a ROI box and submit an *Inside Query*. The database will return the neurons or neuropils which are completely enclosed within the ROI box.

d. ***"Intersection" Query***: An interesting challenge for neuroscientists is to locate the neurons which connect through a specific region of neuropil or neuron. NNG's *Intersection Query* allows the user to find the neurons passing through the intersecting region of two objects. For example, the alpha and alpha's lobe of the mushroom body may have different functional significance in terms of



(a) The user selects a starting object, “dlp” in this example, and then clicks on it to send a query. The left side of the display is the list of neurons that connect to dlp. (b) The user selects one neuron from the list. (c) The user defines a terminal box. When the user hovers the mouse cursor above the terminal in Innervation mode, a terminal box appears. The user can adjust the size of the box using the mouse or keyboard. (d) The user queries the connecting neuron passing through the terminal box and selects the next desired neuron from the results. (e) The user defines the other terminal box for the next query. (f) This time, the user queries for connected neuropils and neuropil “AL” is returned. The query sequence is listed on the right side of the display.

Fig. 9. The sequences for the Innervation query operation.

memory processing and storage [25]. The user may use the ROI box to enclose regions of the mushroom body in part or in whole. The query will only return the neurons passing through that intersecting region enclosed in the ROI box and the target mushroom body. (Fig. 7)

e. ***“Across” Query***: In the aforementioned query techniques, queries often result in return of too many clustered neurons from the database. When this happens, the user may have difficulty identifying the spatial distribution of the neurons or finding specific neuron (i.e. one that passes through a specific location). In this case, the user can further filter the query results by specifying an across box. As a result, NNG will only show the neurons which pass through the box. The user can use the mouse to move and adjust the shape and size of the box. The shown neurons that pass through the across box are updated in real-time according to the new location and cover region of the across box (Fig. 8).

f. ***“En route” query***: This query is useful when the user wants to find the mid-way point connection between two objects. For example, say there are three objects, O_1, O_2, and O_3. When the user wants to know the route from O_1 to O_3 going through O_2, he can perform an *En route query*. The *En route query* actually performs two queries and returns the union results of those two queries. The user can also achieve *En route Query* using a combination of Boolean Queries. The following shows the formula of *En route Query*:

$$((O_1 \text{ AND } O_2) \text{ NOT } O_3) \text{ OR } ((O_3 \text{ AND } O_2) \text{ NOT } O_1)$$

g. ***“Innervation” Query***: *Innervation Query* is a novel querying technique that allows the researcher discover the neural circuit path within the fruit fly brain step-by-step. The user begins the *Innervation Query* by selecting a starting object and then sends the query by clicking the object on the screen. The query results are then shown on the screen and the user can select the next query object from the set of connected objects of the results. NNG allows users to quickly preview each resulting neuron by passing the mouse cursor over the list. This helps the user decide which one to choose next. The user can also specify the region around a particular terminal of the neuron when the mouse cursor hovers over that terminal. A terminal box centered on the selected terminal will appear which allows the user to query the neurons passing through that terminal box. By repeating these steps, the user can reach the target object. NNG helps the user build neuron circuit pathways step-by-step and one-by-one (See Fig. 9. and supplemental video for demonstration). All innervation query operations are performed interactively on the screen.

5 IMPLEMENTATION

NNG’s user interface was developed using Java and SWT [26]. The 3D scene is rendered by OpenGL (JOGL) and GLSL. Taking advantage of Java’s many features and benefits, NNG is a cross-platform application which has been tested in both the Windows and Linux 32-bit and 64-bit environments. The 3D Neuron Image database is a MySQL database which can reside in a remote location and communicate with the client-site application over the Internet, hence reducing the workload of the client’s machine.

This system also allows groups of neuroscientists from around the world to work together to analyze the same data, NNG will be accessible via a web interface.

6 RESULTS AND DISCUSSION

In this research, we developed a novel application that integrates a visual interface with a 3D neuron image database. This new development combines the strengths of a friendly visualization interface and the power of query-based database operation which could help neuroscientists uncover information pathways within thousands of neurons (see supplementary videos for demonstration). Using fruit-fly brain images, while still of substantial research value, might only be the starting case: in future research, various neuron image datasets using unified coordinate space can be used by NNG, for example, that of a bee or a mouse.

BrainGazer developed by Bruckner et al. [12], has provided several basic query techniques. Their Path Query allows the user to sketch an arbitrary path using the mouse cursor on top of the visualization and obtain nearby objects immediately. However, the user is not able to specify the path between two specific regions. NNG's AND operation allows the user to select two objects (which can be neuropil, neuron, or ROI box) and find the connecting neurons between these two objects. When the across box is enabled, only the neurons passing through the specific regions will be shown. NNG can find the connections not only by volume, but also by terminals. A combinatorial graphic management together with Boolean Query technique provided by NNG also gives users the previously unseen flexibility to narrow down the query results. Another unique feature of NNG is its ability to find a hypothetical information pathway within the fly brain step-by-step. Typical use of the Innervation query is shown in Fig. 9.

The handling of volumetric images is extremely computationally demanding. When developing NNG, the main concern we wanted to address was how to be able to easily manipulate and analyze more than 10,000 neuron image data sets in an efficient manner and how to handle the growing amount of data collected. To address all of these concerns, we implemented the tracing line representation in NNG. Tracing lines provide a simple and fast way to render neurons (although still approximate). We compared the terminals derived from the tracing lines with the results from the real volume data and found the terminals on tracing lines are greater than may be discerned visually from the volume. Therefore, we have built in a tolerance (i.e. a size of the unit space) when creating the database. If the user wants to verify the results in the original volume data, we include a link to call an external volumetric image viewer. Though the Innervation query is useful, it only allows users to specify the starting object, after which they must locate the target object step-by-step. In the future, we will integrate a path-finding algorithm to address this issue and, as a result, save the user much time and effort.

The handlings of the female and male neuron images are separate. Currently, the 3D neuron image database stores information from about 10,000 female fruit-fly neurons and 3,000 male fruit-fly neurons. The figures and examples used in this paper are from the female data. The user can specify whether they want male or female data in the program argument and NNG will load different datasets and database tables accordingly. The ability to locate and compare corresponding male and female fruit-fly neurons is ongoing. Currently, we are looking for workable algorithms for the geometric transformation between the male and the female fruit-fly brain space.

The reliability and the significance of the neural connectivity in NNG rely exclusively on the image accuracy in the preprocessing stage (e.g. 3D warping). After a batch processing job warps thousands of neuron image sets into the standardized fruit-fly brain, NNG can easily identify mis-warped neurons. When looking into the terminal distributions of NNG, an out-of-the-brain border terminal might be representative of an error.

The application of the graph theory to the analysis of neuron connectivity is an emerging research topic recently [27]. Once the database has been established, we are able to locate neighbors of all neurons and neuropils. One of our next objectives will be to build the correlation maps between the neurons for graph analysis.

Currently, the system does not contain data on dendrites and axons, thus, the connections between the neurons are un-directional. Dendrite and axon information will be available in the near future and we have reserved the directional flag for this in the database already. With directional information, the neuron information pathway will be more realistic in terms of functional significance.

NNG has also incorporated a web-interface that allows researchers from all over the world to access. NNG is now integrating with another fly brain image database also, both of which will be publicly accessible once completed (Anyone who may be interested in NNG, please go to: http://211.73.64.34/NNG to register).

NNG is the result of an inter-disciplinary collaboration between computer scientists and neurologists. Experts in theoretical neural computation will become involved in its further development in the future. Considering its current achievements and future developments, the Neuron Navigator will continue opening doors to new and different areas of research.

7 NEUROLOGICAL INSIGHT

The significance of NNG to neuroscientists is that it provides a flexible apparatus to investigate the structure of neural network with an ensemble of single neurons in the whole brain, currently for *Drosophila*. For example, with the "across" search, it is simple to retrieve long range commissural tracts connecting the medulla (part of the optical lobe responsible for processing visual signals) on both sides of the brain (Fig. 10). These commissural tracts have been known since 1976 in studying the anatomy or development of the central nervous system in *Drosophila* and other insects [28] but no extrapolation of the circuit has been made due to the difficulties in visualize more than one neurons in one anatomical study in 3D, let alone to reconstruct them from separate observations. With the aids of NNG, it is quite straight forward to focus on one particular commissural tract and then to find out other neurons connected with the terminals of the tract (Fig. 10). These neurons communicate through the commissural tracts and may be responsible for the reciprocal modification of visual singles for the proper weighing of signals coming from different directions. Further electrophysiological of genetic investigations may precede on those NNG identified candidate neurons up/down-stream the commissural neuron for their real functional significance. Eventually, NNG may become a computer-aided test board for neural circuitry.

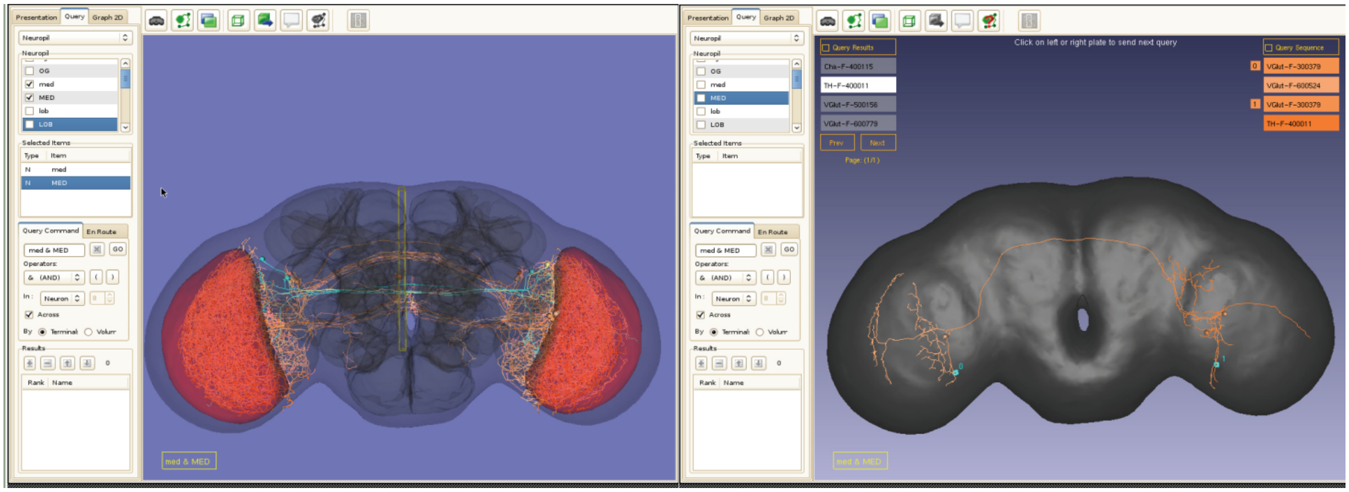


Fig. 10. Many long range commissural tracts may be found between medullas with the "across" search (9(a)) on two sides of the Drosophila brain. The dorsal most one may be selected by restricting the "across" box and then neurons connected through its terminals may be found and selectively visualized in the viewer (9(b)). Blue cubes represent the connection sites of the commissural neuron with other neurons.

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