Case Study: Investigating Dendritic Spines Using Computer Visualization Techniques

Dennis Jen[1], Peter Parente[1], Jonathan Robbins[1], Christopher Weigle[1], Russell Taylor II[1], Alain Burette[2], Richard Weinberg[2]

[1]*Department of Computer Science, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*
[2]*Department of Cell and Developmental Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*

Abstract

This paper describes ImageSurfer, a volume-visualization system aimed at the study of nerve-cell dendritic spines imaged using confocal microscopy. ImageSurfer's final design can create convincing visualizations capable of answering our specific scientific questions through its four-window interface, featuring a volume and surface rendering, a 2D graphical-user interface, a magic cut plane with a height field, and a graph. Furthermore, this paper describes the three design iterations that led to ImageSurfer's final design, the lessons learned through the design process, and encouraging informal feedback. Through the informal feedback, users reported that ImageSurfer enabled them to explore more dendritic spines than in prior techniques and understand the layout of where proteins of interest lay with respect to the dendritic spines.

I. Introduction

Confocal microscopy has given scientists a powerful technique in obtaining high-resolution images of biological structures. By utilizing the power of the human visual system, scientific visualization offers scientists an intuitive tool in interpreting data. This paper describes ImageSurfer, a volume visualization system that we have developed to answer specific scientific questions for two cell biologists, Dr. Burette and Dr. Weinberg.

This paper is organized in the following fashion: In section 2, we describe this problem domain followed by the scientific questions that ImageSurfer sought to answer in section 3. Section 4 provides a description of the data collection and the characterists of the data. In section 5, we describe two visualization systems capable of visualizing our datasets. Through close collaboration with the cell biologists, we followed an agile design methodology, resulting in three design iterations. We describe our design choices leading to these designs in section 6. In section 7, we document the informal feedback we garnered from the biologists. Furthermore, we describe ImageSurfer's implementation in section 8. Finally, in section 9, we describe future plans for improving ImageSurfer.

II. Problem Domain

Cells called neurons transmit information through the nervous system of an organism using electrochemical reactions. When a neuron fires, it sends an electrical impulse down a long arm-like structure called an axon. The impulse travels to the end of the axon where it sets off a chemical transfer. The chemicals from the sending neuron diffuse across a gap to small receivers, named dendrites, on neighboring receiver neurons. The receiving neurons then fire their own electrical impulses in order to propagate the signal.

Tiny structures (0.5-2 μm length, 0.1 μm diameter) called dendritic spines are located along the length of nerve cell dendrites. These small bodies protrude from a dendrite and are shaped like miniature light bulbs with distinct head and neck regions. Research suggests these spines play a role in insulating specific regions of the dendrite, as a result signaling molecules from neighboring neurons [7]. Consequently, this might even be the basis of memory at the organismal level [1,3,8].

Dr. Burette and Dr. Weinberg are currently engaged in a study of dendritic spines. Specifically, they are interested in determining where and how a protein called plasma membrane calcium ATPase (PMCA) concentrates within these structures. Understanding this connection could provide insight into how signal chemicals are released back into the synaptic connection after use.

III. Scientific Questions

Through the problem domain and the research interests of the scientists, we have defined two specific questions of interest. These questions act as guidelines for the development of the visualization system:

1. How is the concentration of PMCA distributed within dendritic spines?

2. In a given spine, where is the greatest concentration of PMCA? Where is the least concentration?

The first question places an emphasis on relating concentrations of PMCA to their locations in space.

Creating a tool that helps visualize this spatial relationship is the primary goal. The second question seeks to relate parts of a PMCA distribution. Aiding the scientists in understanding the variation of PMCA within a region is the secondary goal of the visualization tool.

IV. Data

A section of nerve cells from the brain of a 28-day-old Sprague-Dawley rat is stained using a primary antibody against PMCA that fluoresces. Additionally, the sample is stained using a different fluorescent called DiO that helps identify the lipid layer of the neurons in the sample. The tissue is then incubated for two days.

In this section, we describe how data is collected from the brain, followed by a description of the characteristics of the data collected.

4.1 Collection

After the two-day incubation period, images of the sample are taken using a charged-coupled device (CCD) camera attached to a confocal microscope [2]. The sample is illuminated with a certain wavelength of light that causes the DiO to phosphoresce as an image of a single slice is captured. The sample is then illuminated with another wavelength of light that causes the PMCA antibody to phosphoresce as an image of the same slice is captured. This process is repeated for each slice in the sample stack, yielding two images for every slice. The resulting images show the locations and concentration of PMCA proteins as well as the general structure of the nerve cells through the DiO staining.

Two characteristics of this data collection impacted our design. First, the limits of the optical resolution of the confocal microscope results in diffraction. Consequently, it becomes difficult to distinguish small objects [6]. [11] suggests that these imperfections can be removed by applying deconvolution algorithms. Second, a great deal of background noise exists in all of the images, originating from the preparation method of the tissue samples. Each sample contains a number of significant features besides those of interest to the researcher. The PMCA dataset is cluttered with splotches of light coming from PMCA proteins that are not associated in any way with the neurons. The DiO dataset contains many other biological features such as blood vessels or parts of other neurons that can obstruct the view of the dendritic spines of interest. Unfortunately, these sources of interference cannot be removed easily during sample preparation, and, therefore, must be accounted for in our visualization software.

4.2 Characteristics

The images captured during a given run of the experiment are 2D slices of a 3D space. During a single run of the experiment, the confocal microscope images each slice twice. As a result, two datasets are produced, each containing a number of 2D images corresponding to the slices of the 3D tissue sample. ImageSurfer reconstructs the original 3D space that the images collectively represent.

The data values are structured in a regular grid pattern within any given 2D image. The camera attached to the confocal microscope simply images the light emitted from the prepared tissue sample as it hits the camera's CCD. The point samples of light intensity are then discretized into a set number of pixels (currently 1024 by 1024 pixels). These pixels form a common x vs. y grid image. No other filtering is performed on the data before it is collected, and no averaging or other transformation is applied to the point samples collected by the camera.

Each data element within a given image takes the form of a single scalar value. Any scalar value at a location of (x,y) within an image represents the intensity of the light that struck cell (x,y) on the CCD camera during the experiment. Since the measure of intensity of light is based off an absolute ground level (i.e. zero is equivalent to no light), the scalar values are classified as part of a ratio scale. The 8-bit quantization method for collecting intensity values in this experiment constrains the scale to a range of 0 to 255.

As mentioned previously, the data contains noise from both imperfections in the optics of the microscope as well as noise from left-over biological structures in the sample. There are no missing values in the sampled volume.

V. Related Work

A number of visualization systems are available that can be used to visualize the PMCA and DiO datasets separately. Generally, these systems apply different volume rendering techniques to reconstruct 3D volumes. Users may explore the volume through rotating, zooming, and panning. However, these systems do not enable users to visualize the interactions between multiple datasets. In this section, we describe two visualization systems: VisBio, an open-source system, and ViewVol, a proprietary system.

5.1 VisBio

VisBio is a visualization system built using the Java programming language and uses VisAD for its visualization capabilities [4]. The primary goal of this system is to visualize multidimensional datasets and provide tools for analysis. To achieve this goal, the system provides some of the following notable features:

- Color mapping
- Volume rendering
- Arbitrary angles slicing
- Measurement tools
- Cell drift correction

In addition to the aforementioned features, VisBio provides analysis tools for stacks of image that vary over focal plane (like the DiO and PMCA datasets) and stacks of image that vary over time.

5.2 VolView

VolView is a proprietary visualization system built using the Visualization Toolkit (VTK) for its visualization capabilities [5]. Like VisBio, the VolView system is interested in visualizing stacks of images as volumes. Unlike VisBio, this system does not offer tools specifically for analyzing image stacks varying over time. However, some of the notable features that VolView does provide include the following:

- Loading of multiple file formats
- Color maps and transfer functions
- Multiple volume rendering techniques
- Surface rendering
- Animations creation
- Cropping and cut plane
- Image processing filters
- Distance, surface area and volume measurements

VI. Design

During the initial design of the system, we investigated a number of techniques suitable for answering the scientific questions stated in section 3. We combined the most appropriate of these techniques for implementation in an initial prototype. After demonstrating the prototype to the scientists and our peers, they offered feedback, which we used to implement improvements to our visualization. Finally, after giving the scientists months of using the software, we implemented new features and improvements.

6.1 Initial Design

The visualization uses two windows to display the datasets. In the first window, the density values of the DiO dataset are shown using a direct volume rendering technique. The transfer function for the volume density values is set so that the nerve cells appear with nearly solid surfaces. The surfaces are both lit and shaded to reveal structure better. Density values in the volume are mapped to a simple grayscale colormap. The entire volume is enclosed in a set of axes that indicate the width, height, and depth of the volume in the units of the original sample (μm). The grayscale rendering is placed over a light blue background for contrast. The view of the volume is translated, rotated, and scaled using a mouse.

A red plane is placed inside the DiO volume rendering with a cone glyph at one corner of the plane. Users move, rotate, and scale this plane within the volume using a number of slider controls. Once users position the plane over a slice of interest in the DiO volume, clicking a button reveals a slice at the same position, orientation, and scale in the PMCA dataset. This tool can be thought of as a cross between a cut plane and a magic lens: a magic cut plane.

Using the magic cut plane, users select a slice of PMCA, which in turn is rendered in the second window as a height field. The concentration of PMCA is redundantly mapped both to the height of the surface and to a color in a perceptually ordered scale. A cone glyph is placed at the proper corner of the height map so that it can be aligned with the glyph used in the first window. Axes are provided around the height map to show appropriate values for the height, width, and length of the surface. A legend for the color scale is also provided to map its colors into numeric intensity values. Again, the view of the entire volume can be translated, rotated, and scaled using a mouse.

A third window provides control over the renderings and cut plane. The transfer function for the DiO volume rendering as well as its rendering quality are configurable in this window. Options for setting the scale and sampling rate of the PMCA height field are also provided here. Additionally, the sliders for controlling the position, orientation, and scale of the cut plane are shown in this window.

6.2 Justification for the Initial Design

In an ideal world, the scientists would be able to simply select and peer into dendritic spines of interest within a 3D rendering of the DiO dataset in order to see the PMCA concentration inside. Unfortunately, transparency is needed to see inside the spine, while occulsion is needed to allow the spine of interest to block out other regions of the screen. Due to the conflicts between these two properties, this approach is not feasible. To avoid this problem, the inital design attempts to retain the sense of selecting a spine of interest and viewing the PMCA concentration within that spine, but accomplishes it by simplifying and splitting the view of the data to two separate renderings.

In the first window, the DiO is rendered using a composite direct volume rendering technique in order to construct a view of the data that is natural and familiar to the scientists. The entire volume rendering is lit, shaded, and colored according to a grayscale colormap in order to display shape and structure information. Emphasizing the shape and structure of features in the volume provides the scientists with a clear view of the dendritic spines. Rendering only the DiO volume in this window instead of merging the DiO volume with the PMCA volume also helps to reduce clutter and allows the scientists to focus only on finding dendritic spines. The axes provided around the entire volume provide a global frame of reference for locations within the volume.

The magic cut plane is placed inside the DiO volume and acts as a selection tool. It is colored bright red to allow it to be quickly spotted. Users position, orient, and scale the

plane in order to slice through a region of interest to reveal the underlying PMCA concentration in the second window. The use of a plane to select a slice of interest through the DiO rendering, most likely through a dendritic spine, effectively reduces the dimensionality of the data and opens up the possibility for using well-defined 2D visualization techniques to display the DiO and the PMCA simultaneously. While selecting a 2D instead of a 3D region does sacrifice the ability to view all of the PMCA in an area at once, it does provide a simple view of the PMCA in relation to its location within a DiO slice. To give users a feel for the orientation of the height field with respect to the plane, the cone glyph is placed at the corner of the plane and is mapped to the orientation of the height field in the second window.

In the other render window, the PMCA along the magic cut plane is drawn as a colormapped height field. The height of the field allows the scientists to compare relative concentrations in the PMCA distribution while the perceptually ordered colormap redundantly emphasizes the concentration levels. The axes provide a frame of reference for the height field in global units, while the cone allows the orientation of the height field to be mapped to the proper orientation within the DiO volume rendering.

6.3 Second Design

The initial design presents users with the ability to select and explore PMCA concentrations of interest. The concentration values are presented as a height field that helps viewers compare values across a selected slice of the volume. While this design helps to answer part of the scientific questions, it fails to provide information about the location of PMCA within DiO without forcing users to shift their focus back and forth between two windows. For instance, when a scientist selects a dendritic spine of interest, the magic cut plane is positioned so that it slices through the spine. The rendering of the PMCA height field appears in the second window, detailing the concentration across the selected slice. In order for users to determine where the concentration is greatest and least along the slice, they must first figure out how the height plane is oriented in space according to the camera view in each window. The cone glyph provides a little assistance in this task, but, nevertheless, users are forced to derive the relationship on their own.

During the design process, we explored alternatives and improvements in hope of removing this shortcoming. One slightly different approach is to eliminate the second window altogether and to show the PMCA concentration directly inside the DiO volume rendering. In this design, the magic cut plane still acts as a selection tool, but also becomes the PMCA height field itself. For instance, users again select a dendritic spine of interest using the plane. Once users select a spine, the cut plane warps into a height field detailing the concentration distribution within that spine. This approach promises to relate the PMCA values directly to their location in the dendritic spine as the height field is rendered directly inside the DiO volume. While the idea sounds good in theory, it also introduces the possibility of confusion. Rendering a height field inside a 3D volume causes a conflict between the use of the third dimension for spatial relationships in the DiO and the use of the third dimension as an indicator of concentration in the PMCA, not to mention likely possibility that the the height field will occlude the volume and vis versa.

Another idea eliminates the height field and the magic cut plane in favor of coloring the DiO volume according to PMCA concentration. In this alternative, the opacity transfer function for the volume rendering continues to act on the DiO values to create a rendering of the neve cell structures of interest in the volume. The color transfer function, however, now acts on the PMCA dataset scalar values in order to color the nerve cell structures in the volume according to the PMCA concentrations they contain. This technique directly correlates the PMCA values to their location in the DiO dataset. In addition, it is capable of showing the concentrations in the original dataspace without any work on the part of the scientists. However, it does sacrifice two features of the intial design. First, coloring the volume rendering only shows composite PMCA concentrations. The opacity set by the DiO density at each voxel allows concentrations of PMCA at the back of the volume to ‘shine through’ to the front. This occlusion problem introduces error into the perceived color of the PMCA, making it difficult to judge relative and absolute concentration values. The issue could be removed by rendering the volume more like a solid isosurface, but then only the concentrations along the outside of the surface are visible at any one time. Second, using color for relative PMCA value comparisons, like those required to understand how the PMCA is distributed within a dendritic spine, is not as effective as using height. The use of color or texture in this approach only provides a very gross estimation of how the PMCA varies across a spine.

A third approach for improving the correlation between PMCA concentrations and their locations within dendritic spines was suggested by our advisor, Dr. Russell Taylor, and requested by the cell biologists. This idea agrees that the magic cut plane and height field from the initial design are appropriate tools for exploring relative and absolute concentrations. However, instead of redundantly displaying PMCA concentrations with a height field and colormap, only the colormap on the height field needs be changed to reflect DiO concentrations. For instance, positioning the magic cut plane through a dendritic spine in the DiO volume reveals the PMCA concentration along the slice in the second window as well as a colored region representing the DiO along that slice. This coloring technique results in a rough outline of the spine shape. In effect, this technique reverses the problem of locating the

PMCA inside of the DiO and instead attempts to show where the DiO is located on the PMCA height field.

Out of these alternatives, the last one appears to improve the initial design in the best manner possible. Not only is the change simple, but it also has a minimal effect on the other good aspects of the original design. To integrate the change, a colormap ranging from white for low DiO values to red for high DiO values is used to color the height field of PMCA concentration. The use of the red color prevents masking of the high-frequency changes in the height field as well as draws attention to regions inside the dendritic spine. The entire rendering is placed on a black background to provide contrast against the colored height field.

6.4 Third Design

While improving the initial design, the second design still forced users to shift their focus between the two windows and did not give an overall view of where PMCA is within DiO. The second alternative, in section 6.3, in combination with the second design as whole would provide the users with this overall view of the both datasets while still allowing users to use the magic cut plane to investigate spines of interest. Thus, in addition to a volume rendering, we provide a solid isosurface rendering that can be colored based upon PMCA concentrations—a colored isosurface. Although only concentrations on the surface are visible, users acquire a preview as to where PMCA is concentrated, before they choose a spine to investigate more closely.

In addition, since a non-grayscale colormap can be used to color the isosurface, we changed the background of the volume display to black instead of light blue. This allows a range of colors to be seen against the dark background.

According to requests from the scientists, we've added an initial tool for statistical analysis, called a spline tool. Similar to the magic cut plane, this tool allows users to focus onto a smaller area of the visualization. After users position the spline onto the height field, intensity values that lie along the spline are captured and displayed as two overlapping plots along a two-dimensional graph. The plots represent either concentration of DiO or PMCA along the point-sampled spline.

6.5 Lessons Learned

We used an agile software development methodology in constructing the visualization, which enabled us to redesign multiple times. Accordingly, we continuously changed our plan based on constant feedback from the clients and others. Over the course of development, the initial design evolved into a more robust system based largely on this feedback from collaborators and peers. Constant contact with the cell biologists enabled us to resolve issues, determine the usefulness of certain functions, and plan new features with ease. Our willingness to seek aid from our advisor and to heed feedback helped us discover design alternatives. As a result, the final design chosen for implementation appears to be the most applicable software product for the visualization goals. If given the chance to design the system again, we would wholeheartedly choose to follow the same approach. In effect, we planned for change and benefitted from it.

VII. Informal Feedback

By meeting with the researchers often, we were able to ensure that the visualization system answered the scientific questions and fulfilled their requirements. Figure 1 is a picture of Dr. Weinburg using the second iteration of the ImageSurfer.

Unfortunately, the researchers have not had a chance to use ImageSurfer extensively in their research yet. However, they have had the opportunity to review and comment on the chosen techniques and implementation. The researchers commented that the system allows a user to "cut a dendrite from any angle." In their original method of exploring the spines, the researchers would alternate between an image of the DiO and followed by an image of PMCA, seeking dendritic spines oriented entirely within an image plane. In any given dataset, approximately four or five dendritic spines are situated in this ideal orientation out of a myriad of others. Thus, our new magic cut plane tool allows the researchers to explore spines at any orientation, drastically increasing the wealth of information in each dataset.

Figure 1: Dr. Weinberg using the second iteration of the visualization system

Another remark made by the researchers was that they had "never seen height used to display concentration values before." Traditionally, they have relied on comparing color values to determine relative concentrations of proteins. Our approach, on the other hand, allows the scientists to compare heights instead, which is an easier task for the human visual system. More accurate qualitative conclusions about the concentration and location of PMCA are possible using this alternate technique.

A particularly popular feature is that of setting the focal point. By doing this, rotations occur about the focal point rather than the center of the visualization. When investigating a spine of interest, it has been useful to set the focal point at the neck or head of the particular spine and rotate about that point on the spine.

In addition, the researchers commented that the newest technique of coloring the dendrite based on the concentration of PMCA provides a "rapid understanding of the layout of the PMCA." They agreed that this was a convincing way to portray the distribution of PMCA. Despite being convinced of this, the researchers thought their colleagues might be more skeptical of the relationship depicted in images of the colored isosurface. However, they included images of the colored isosurface in a presentation at the *Sixth IBRO Congress of Neuroscience* in Prague and were pleasantly surprised when they were able to convince attendees of the relationship.

VIII. Implementation

ImageSurfer is constructed in the Java programming language. The Visualization Toolkit (VTK) provides the necessary visualization capabilities, and makes use of OpenGL for its low level graphics functionality. The Java Swing package adds a graphical interface and interactivity to the system. The system runs on the Windows operating system, although none of the libraries used are specific to Windows. No special hardware is required to run the program beyond a decent computer by today's standards (1.2 GHz, 512 MB RAM, 3D accelerated video card). All input to the program occurs using a standard mouse and keyboard.

8.1 Interface

ImageSurfer's interface is divided into four main windows as shown in Figure 2. The control window, houses the widgets that control the visualization parameters. The Volume window displays the DiO volume or, in the case of Figure 3, surface renderings and allows users to move the camera using the mouse. The Magic Cut Plane window displays the PMCA height field rendering and allows users to move the camera using the mouse as well. The Graph window displays the graph of the DiO and PMCA along a spline.

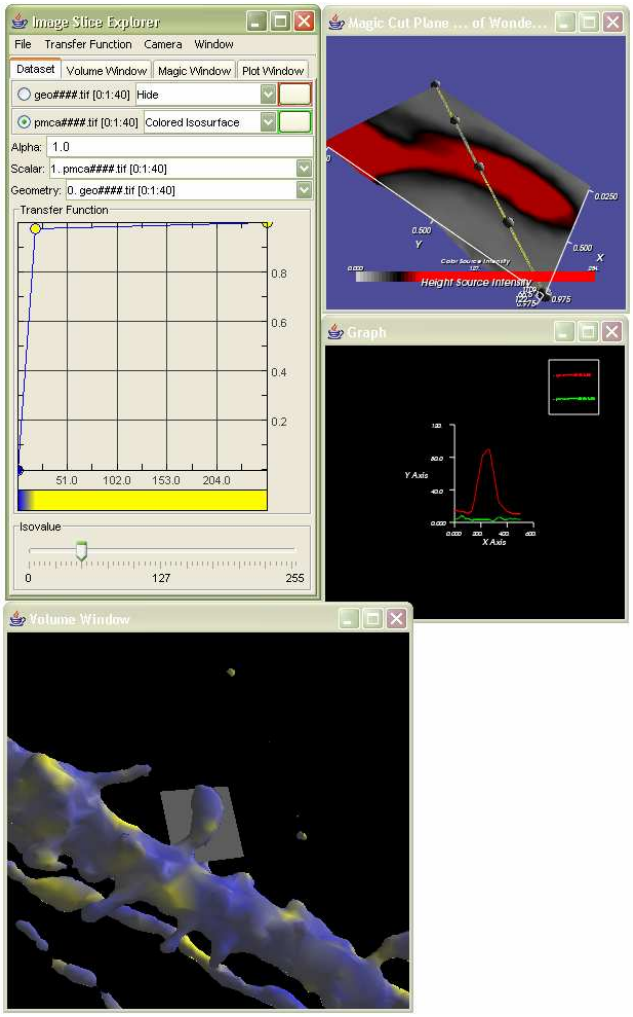

Figure 2: Four program windows

A number of options and commands are accessible through the control window shown in Figure 3. Most of the options are configurable using slider widgets, menus, drop down lists, list boxes, and text boxes. All told, the options available in this window allow users to do the following: open datasets, save images, save image sequences, save and load transfer functions, save and load camera positions, set the voxel dimensions, interact with the transfer functions and color maps, position the magic cut plane, capture the magic cut plane data to a height field, capture the spline tool to the graph, reset the camera position, select the rendering technique, select a region of interest, and label the graph.

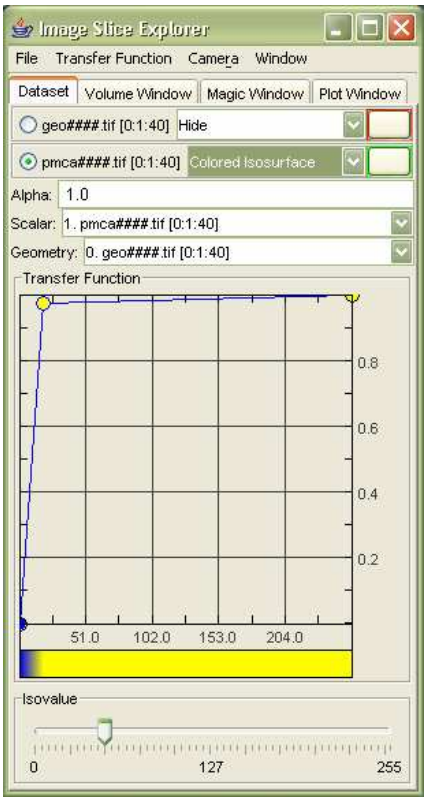

Figure 3: Control window with Dataset Panel displayed

The Volume windows shown in Figure 4 provides a view of the nerve cell dendrites found in the dataset as a surface in one window and in the other window as a volume. Axes and the magic cut plane are rendered along with the rendering. By using the mouse with the left, middle, or right button, users can rotating, panning, and zooming the camera respectively. Within this window, users may move, size, and orient the magic cut plane within the volume rendering also. However, in order to manipulate the magic cut plane, you need to first mark a check box in the control window.

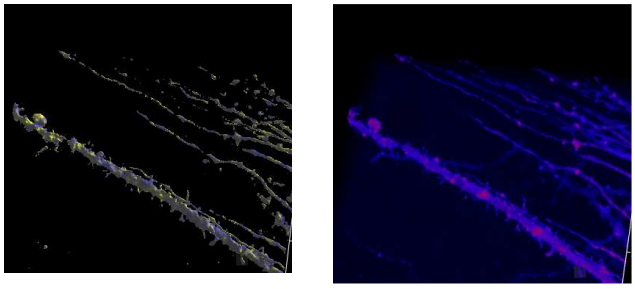

Figure 4: Surface and volume rendering of DiO

Next, the Magic Cut Plane window offers a view of the PMCA distribution along the magic cut plane shown in Figure 5. The intensity values of PMCA are mapped to a height field while the DiO values are shown using a color scale set by the user. This window allows a user to view relative concentrations across regions of interest in one location. Once again, users can rotate, pan, and zoom the camera by using the mouse with the left, middle, or right button pressed respectively.

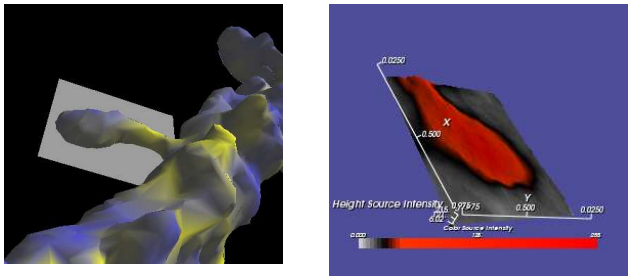

Figure 5: Colored isosurface rendering of DiO and associated height field rendering

Finally, the Graph window offers users an even more restricted view of the region. Using the mouse, users can position the spline by moving the nodes of the spline, represented by the gray balls. After the spline is positioned, the software can capture the concentration DiO and PMCA along the spline and display a graph shown in Figure 6. In the Graph window, the DiO is denoted by the red line, while the PMCA is denoted by the green line.

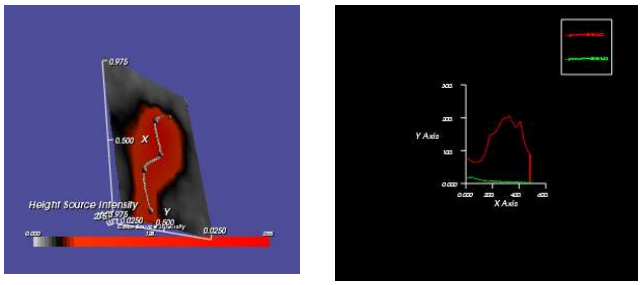

Figure 6: Height field rendering with spline tool and associated graph rendering

8.2 Packaging and Deployment

In order to ease the installation of the software by the scientists, the Java class files are packaged into a jar file then wrapped into an installer. Constructed using the Qsetup Installation Suite, the installer manages the installation of the various VTK libraries, jar files, and environment variables. This simple addition to the project eases the burden of installing and uninstalling the software in addition to performing upgrades. An online user manual is also available to further assist users.

8.3 Lessons Learned

The process of implementing this visualization has served as an excellent source of information about the collaborative design process between clients and developers, and agile software development. Using an object oriented approach in an interpreted language helped us quickly integrate suggestions from the scientists into the system, or even junk certain components to be replaced with new ones. VTK assisted in this area as well with its pipeline model. We added, modified, or replaced segments

of the pipeline as needed with only minor changes to other neighboring segments. The tremendous number of pre-built visualization components offered by VTK and the excellent VTK community support also aided us in rapidly developing and testing versions of the visualization. All of these factors assisted us in rapidly evolving ImageSurfer towards an optimal solution.

Providing a user-friendly method for manipulating the magic cut plane proved much harder than expected. At first, an attempt was made just to move, rotate, and scale the cut plane using mouse manipulations. This approach was quiet unwieldy as it was difficult to manipulate the plane in a 3D volume using the mouse on a 2D surface. Our next approach was to use sliders to control the position and size of the cut plane thus making selection of a slice of interest more manageable, but are still time consuming to use. In the current implementation, we've returned control to the mouse, but the added feature of setting the focal point onto a spine of interest has made positioning the magic cut plane easier, but still unwiedly to an untrained user. If we were to implement the system again, finding a better method of control over this plane using a 3D input device, a more intuitive mouse control system, or even a different slice selection technique would be of greater importance earlier in the development process.

IX. Future Work

The next step in improving the visualization will be to develop a data export tool for statistical analysis. The scientists have articulated that in order for the software to be of long-term use in their research, it must be able to help them determine relationships of statistical significance over a large population of spines. The scientists would like to be able to export concentration values running from the head to the neck of many dendritic spines, and analyze the values using other software packages. We've begun this process through the creation of the spline tool, which allows a user to select a 1D segment of PMCA and DiO values for export from our height field rendering. We will continue to develop additional tools by enabling users to capture concentrations of PMCA per area and then to concentrations of PMCA per volume. By exploring these idea and their alternatives in more detail, we will continue work on this project.

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