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Immersive environment for creating, proofreading, and exploring skeletons of nanometric scale neural structures

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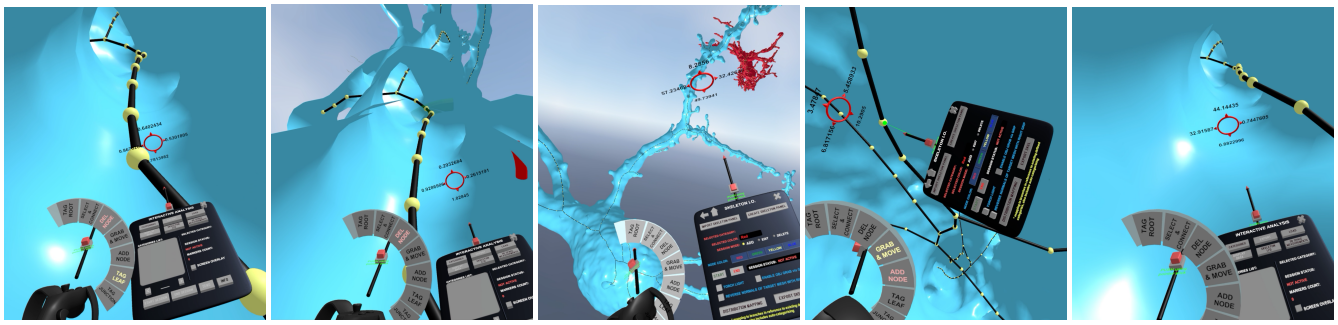

Figure 1: *Our proposed virtual environment enables neuroscientists to immersively create, proofread, and explore medial axis representations or skeletons of nanoscale reconstructions of brain cells. In the example scenario above, skeletons are represented as connected nodes (yellow spheres) and edges (black cylinders), while brain cells are depicted as shaded surfaces (using a light blue color in this example).*

Abstract

We present a novel immersive environment for the exploratory analysis of nanoscale cellular reconstructions of rodent brain samples acquired through electron microscopy. The system is focused on medial axis representations (skeletons) of branched and tubular structures of brain cells, and it is specifically designed for: i) effective semi-automatic creation *of skeletons from surface-based representations of cells and structures, ii) fast* proofreading*, i.e., correcting and editing of semi-automatically constructed skeleton representations, and iii) useful* exploration*, i.e., measuring, comparing, and analyzing geometric features related to cellular structures based on medial axis representations. The application runs in a standard PC-tethered virtual reality (VR) setup with a head mounted display (HMD), controllers, and tracking sensors. The system is currently used by neuroscientists for performing morphology studies on sparse reconstructions of glial cells and neurons extracted from a sample of the somatosensory cortex of a juvenile rat.*

CCS Concepts

• ***Applied computing*** → ***Life and medical sciences; Imaging;*** • ***Human-centered computing*** → *Pointing; Interaction design;*

1. Introduction

The brain cells, together with their processes, are complex three-dimensional structures, and improving the visual understanding of the relationships between morphological features and functional aspects of these cells is of primary importance to neuroscientists. The recent progress in digital acquisition and analysis of biological samples, e.g., brain tissues, is offering unprecedented possibilities of insights for neuroscientists. For instance, automated serial section electron microscopy (3DEM) provides electron micrographs that can reach a resolution of a nanometer per pixel, therefore revealing features ranging from full structural cellular details such as axons, dendrites, and synapses (the so called "neuropil"), to smaller intracellular organelles like synaptic vesicles. However, neuroscientists still require effective tools and applications to handle this large and complex data. Morphology data at nanoscale resolution provide domain scientists fundamental information for understanding neural processes and interaction between cellular structures [CAK*19]. Furthermore, the challenge of making qualita-

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tive and quantitative assessments of complex and visually occluded individual cellular structures, or groups of them, is beginning to attract neuroscientists towards the use of immersive visualization paradigms. Hence, during recent years, various laboratories pioneered the use of virtual reality (VR) in supporting electron microscopy (EM) structural analysis [CBB*16, ABG*18, UKF*17]. However, while previous environments targeted exploratory analysis of brain structures for specific morphology studies [CBB*16], or neuroenergetics investigations [ABG*18], recently, the need for investigating the features of branch-based cell structures, either for quantification and classification purposes [KRS*19], has emerged. Hence, neuroscience laboratories are investing important resources on creating faithful and smooth medial axis representations of brain cells, to be used for various kinds of visual and statistical analysis. To this end, time consuming image-based manual tools [LBA11, SACF*12, SRHE15] are commonly used for tracing neural processes, while more complicated automatic methods for recovering medial axis representations are still in their infancy [TDS*16] and not yet routinely used in for processing brain cells.

In this paper, we present a novel VR system targeted on creating, proofreading, and exploration of skeleton-based representations of nanoscale brain cells surface reconstructions. The system integrates the following components:

- a fast servo-assisted semi-automatic method for creating skeletons of complex brain cellular reconstructions;
- tools for proof-reading (checking, correcting, comparing) medial axis representations;
- exploration tools, e.g., for performing geometric measurements and statistical computations related to cellular structures and their skeletal representations.

The system is currently used by expert domain scientists for analysis of various cells reconstructed from the somatosensory cortex of a juvenile rat [CAK*19]. We report on a preliminary subjective evaluation of the immersive environment performed by domain experts during creation and proofreading of complex medial axis representations, as well as during analysis of organelles distributions.

2. Related work

Our work deals with the application of virtual reality (VR) technologies to neuroscience investigations coupled with the computation of medial axis representations of highly detailed branched cellular brain structures. In the following, we discuss the previous work mostly related to our contribution.

Virtual reality in neuroscience. Due to the ubiquity of desktop systems, most commonly used visual analysis tools in neuroscience are designed as desktop applications [MAAB*17, HAAB*17]. However, more recently, there is general consensus that the use of stereoscopic techniques, e.g., in VR systems, can provide a more immersive way to explore brain imaging data [KZC*17], and that the increased dimensionality provided by stereoscopy is beneficial for understanding depth in the displayed scenery [AGIM10, HPH*14]. With respect to immersiveness, the effect of stereoscopy has been previously evaluated in the context of visual analysis of volume data, particularly for semitransparent volume rendering [LSSB12, AGI*08], isosurfaces [LBS14], confocal volume images [FKW*08], and for interactive graph analysis [AHKMF11, AGIM10, KZC*17]. Successful examples of applying VR technologies to neuroscience investigations include analysis of glycogen distribution related to neural morphologies [CBB*16], systems for tracing neurons in microscope scans of primates' visual cortex [UKF*17], and the use of heat maps for representing absorption probabilities on nanoscale surface reconstructions [ABG*18]. In this work, we describe an immersive environment for performing shape analysis that is mainly targeted on skeleton representations. To our knowledge, it is the first application of a VR environment towards morphological analysis of medial axis representations, particularly of brain cells.

Skeleton-based representation of surface meshes. Medial axis representations or skeletons can be considered descriptors which jointly describe the geometry, topology, and symmetry properties of a shape in a compact and intuitive way, providing a means to capture the essence of a 3D shape [TDS*16]. Automatically or semi-automatically producing accurate skeleton representations is a challenging task. During the last decades, many techniques have been proposed, particularly by the computational geometry community, for different kinds of 3D models. For a comprehensive discussion of the recent methods for creating 3D medial axis representations, we refer the readers to state-of-the-art reports by Tagliasacchi et al. [TDS*16], and by Sobiecki et al. [SJT14]. In general, there is a huge collection of methods to obtain 3D skeletons, which can be classified according to the input representation: mesh-based [TAOZ12, YSC*16, LAPS17, LWS*15] and voxel-based representations [YLJ18]. Since our system is focused on surface representations, we will mostly consider methods that use meshes, even if our system can be considered independent from the method used for obtaining the medial axis representation of the morphology considered. The system has been designed to import skeleton representations coming from different automatic frameworks: for our initial analysis, we considered the Mean Curvature Skeleton (MCS) algorithm [TAOZ12], and the Center Line Tree method [SBB*00], which are implemented in the Avizo [Wes08] framework.

Medial axis representations in neuroscience. Since medial axis representations provide an adequate and convenient description for branched structures, recently, neuroscientists started exploiting them for representing complicated cellular structures, especially neurons. To this end, they derived specific metrics for comparing branched structures, i.e., trees, based on geometrical and topological features [GA15, GHA15, LWA*17]. These metrics are then used for investigating differences and analogies between morphologies or in general for performing identification and classification [BA17, BA18, RCT*19]. Following this philosophy, recently Kanari et al. [KDS*18] developed a classification framework for neurons completely based on skeletons, which is based on specific topological representations, called persistence diagrams. The framework has been successfully used for objective morphological classification of neocortical pyramidal cells [KRS*19]. It has also been integrated into a more general collaborative framework for the analysis and visualization of neuronal morphology skeletons re-

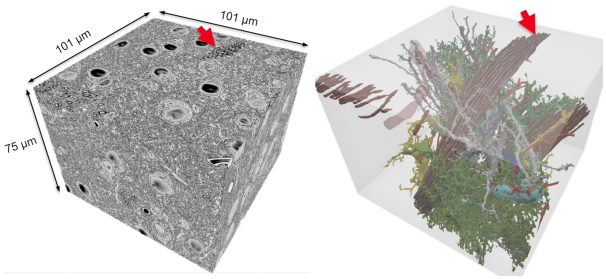


Figure 2: ***Data preparation.*** *Left: we tested the proposed immersive system on models reconstructed from an image stack acquired by serial electron microscopy of a sample from a juvenile rat's somatosensory cortex. Right: sparse reconstruction provides high resolution surface representation of full cellular morphologies.*

constructed from microscopy stacks [AHE*18]. Our proposed immersive environment addresses similar needs, and it is customized for the proofreading and analysis of skeletons of different cells, while leveraging the benefits of a VR system. We believe that 3D branched structures derived by brain cell morphologies can be more effectively analyzed by leveraging cues provided by steroscopy and full immersion which are well suited for 3D scenes. Our framework is general and customizable, and it can be extended to integrate other geometric representations and visual encodings.

3. Application domain: morphology analysis in neuroscience

Before detailing the proposed immersive environment, we first provide a brief description of our particular application domain in neuroscience: the investigation of brain cellular morphologies.

Ultrastructural analysis. Neuroscientists often perform ex-vivo digital acquisition of brain portions by using high resolution electron microscopy systems equipped with high precision cutters [TG16]. This process provides neuroscientists with 3D image stacks representing cellular membranes (see Fig. 2 left). These stacks allow them to recognize and quantify features, such as compounds, synaptic contacts, and even very small organelles like vesicles and endoplasmatic reticulum (ER), at nanometric scale. This imaging technique is becoming even more popular in the field of connectomics, since it enables precise reconstructions of the connections between neurons [LPS14].

Processing pipeline. Given a 3D stack of images acquired by an electron microscope (Fig. 2 left), neuroscientists perform different processing tasks in order to extract relevant 3D shape representations of cellular structures, i.e., surface meshes (Fig. 2 right), that can be used for statistical computations, simulation, or rendering. The process consists of carrying out dense or sparse reconstructions, by using manual, semiautomatic or automatic tools, which label the image pixels in the stack, i.e., assigning them with a unique object identifier, e.g., for axons, dendrites, etc. Currently, we use a hybrid two-step pipeline [CCK*18]. First, a rough automatic segmentation is performed offline through the iLastik tool [SSKH11] - finding the gross features and processes of a cell. This first segmentation is then followed by a manual proofreading phase, performed through the TrackEm2 tool [CSS*12] - specifying exact object boundaries and finer details.

Morphology features. Once the various cells and sub-parts are labelled on a per-pixel level in the image stack, neuroscientists perform various analyses by studying the morphology of the following biological structures (Fig. 2 right):

- **Neurons:** composed of *axons* and *dendrites*, which are the terminals respectively sending and receiving electric signals through *boutons* and *spines*. Boutons and spines are linked and form *synapses*.
- **Glial cells:** neuroscientists mainly focus on *astrocytes*, which are metabolically involved in feeding neurons, *microglia*, which are the main form of active immune defense in the central nervous system by acting as macrophages, and *oligodendrocytes*, which produce the myelin sheath insulating neuronal axons.
- **Organelles:** domain scientists mainly focus on *mitochondria* and *endoplasmatic reticulum*, which are contained in axons, dendrites, and glial cells. They contain the machinery for chemical transformations.

Neuroscientists are interested in studying the relationships between the aforementioned structures, and perform geometric analysis for recovering parameters to be used for simulation purposes or for classification [KDS*18].

Importance of skeletons. Most of the considered cells have complicated branching structures, which are very difficult to analyze using standard mesh representations (see Fig. 2 right). To this end, skeleton representations provide an effective tool for describing them and classifying the various branches, according to the size and the branching level, starting from the soma. For this reason, neuroscientists are increasingly focusing on technologies that can support them in recovering accurate skeletal representations.

4. System overview

The proposed system is a standard 3D VR application with an HMD-based setup using room scale tracking technology. This allows the user to move in 3D space and use two motion-tracked hand-held controllers to interact with the environment, i.e., pointing/selecting objects, and interacting with menus. The system was developed using the Unity game engine.

Scene representation and rendering. The immersive environment provides real-time exploration of scenes composed of surface representations of brain cells and schematic representations of the associated medial axes or skeletons. The level of transparency of surfaces can be interactively controlled in a way to provide context for skeleton exploration. Since the system is also designed for providing endoscopic analysis of cellular processes, a torch tool is provided for shading mesh walls and dark corners during exploration. The tool is attached to one of the manipulators and can be easily used to illuminate dark areas. Basic 3D manipulation options are provided, e.g., object scaling and placement, as well as material and color assignment. Moreover, users can flip the mesh normals, in a way to have a more convenient way of examining the inner/outer

mesh surfaces. With respect to skeletons, the system uses three different representations:

- **sprite-based:** 2D line segments/ribbons represent the whole skeleton geometry (implemented using Unity line renderer module);
- **node-based:** only spheres represent skeleton nodes; depending on the skeleton data, the system can utilize only primary nodes to provide a rough representation of skeletons;
- **complete:** skeleton nodes are represented by spheres while skeleton edges are represented by cylinders.

Algorithm/Tool	Notation/ File Format	Data Type
Centerline Tree (Avizo)	[Point ID , Thickness , X Coord , Y Coord , Z Coord]/ .CSV	Points (file1)
	[Segment ID , Node ID1, Node ID2, Point IDs List] / .CSV	Branches(file2)
Mean Curvature Flow	[x, y ,z] / txt	Points (file1)
	[NodeID1, NodeID2] / .txt	Branches (file2)
	[Sum of points(n) , X, Y, Z, Xn,Yn,Zn] / .txt	Points and Branches
Simple Neurite Tracer (Fiji)	[NodeID, Cell Type , X, Y, Z, radius, ParentID]/.SWC	Points with Branches

Table 1: *Notations/formats used for skeleton data.*

Main features. After loading the cellular morphology, the system enables users to operate on medial axis representations in two modes: *create mode* for creating skeletons from scratch, and *proofread mode* for correcting/editing previously computed skeletons. In proofread mode, the system requires that previously computed medial axes respect specific notations represented in table 1. This notation is valid for most graph representations currently used by most processing software. Specifically, in this paper, we focused on skeletons computed through three methods:

- an automatic volume-based method [SBB*00], implemented in the Avizo framework [Wes08] - it uses connected components for graphs, combining a union-find and a recursive algorithm;
- an automatic mesh-based method [TAOZ12] - it uses iterative contraction through mean curvature flow evolution;
- a manual image-based tracer implemented in the Fiji system [SACF*12].

Our system is able to import and export standard skeleton file formats that are compatible with the previously mentioned systems. It can also be easily extended to support other formats/notations.

5. Interactive tools

Our proposed system provides interactive tools for editing/manipulating medial axis representations. They are summarized in 7 options laid out in an arch-shaped menu (see Fig. 3 left), attached to the left controller. The user can choose one of the options by first rotating his/her wrist between 0 and 180 degrees, and then, once settled on an option, pressing the trigger buttons to select. The options provided by the system are the following:

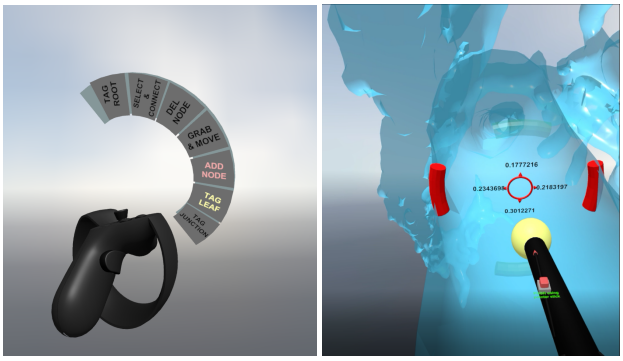


Figure 3: ***Interactive tools.*** *Left: an arch-shaped menu attached to the left controller allows users to select interaction mode with skeletons. Right: a stabilizer servo-assisted tool (in red) guides users through the process of skeleton branch tracing.*

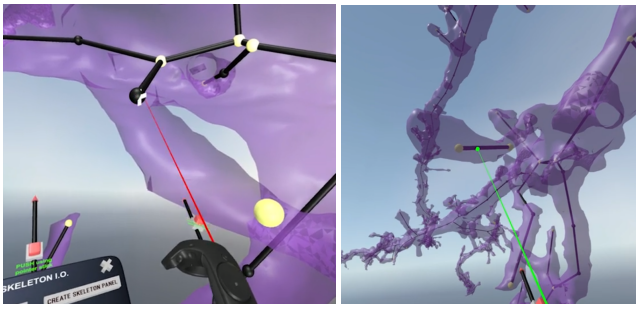


Figure 4: ***Skeleton editing.*** *Our system provides effective tools for rapid editing of skeleton branches. Left: adding connection between nodes. Right: removing a wrong edge from a skeleton branch.*

- **Add Node:** using the trigger button, the user can create a node in 3D space. This process can be fully manual or controlled by a servo-assisted stabilizer. Upon creation, the system automatically pairs up nodes with each other and connects them with an edge, hence, creating a single connected path;
- **Grab and Move:** as part of the proofreading/editing process, nodes can be moved anywhere simply by grabbing them and moving them. This can be achieved thru a combination of an action grab initiated by pressing and holding of the controller's *grip* button while touching the surface of the target node;
- **Select and Connect:** using a combination of point and trigger click, the user can select two nodes subsequently and the system creates an edge connection between them (see Fig. 4 left);
- **Delete Skeleton Element:** the system allows the right controller to shoot a laser pointer by pressing on the controller touch pad. The user can then delete nodes and edges by pointing at a valid skeleton unit object followed by a trigger button click (see Fig. 4 right);
- **Tag Root, Junction, and Leaf:** a similar action of point and trigger at a specified node will save it in its corresponding skeleton file as one of these values: 0=Root, 1=Internal, 2=Leaf, 3=Junction. Tagging a node with "Leaf", "Junction", or "Root" marks it

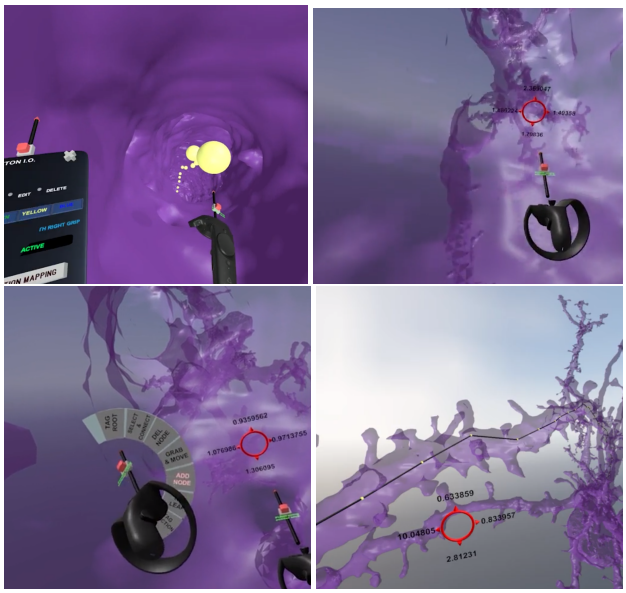


Figure 5: ***Skeleton creation.*** *We propose a semiautomatic and guided method for creating skeletons, based on endoscopic exploration of cell branches, and using a servo-assisted stabilizer.*

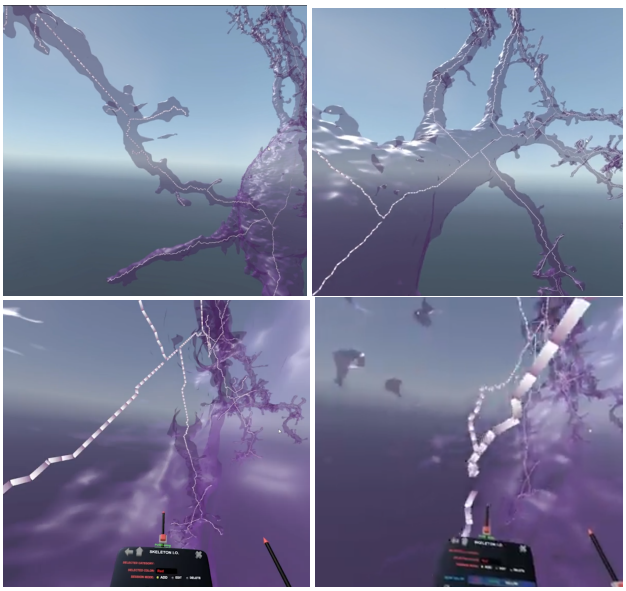


Figure 6: ***Skeleton proofreading.*** *Our system enables domain scientists to perform effective proofreading of skeletons by using endoscopic and external metaphors.*

with a special color material and finalizes the current path as a single branch.

Path stabilizer. The system provides a semi-automatic method for creating skeleton branches through one of the VR input controllers. This method is built around a visual user guide, that operates as reference when tracing the tunnel-like cellular processes through endoscopic navigation. During the exploration of the process, a path stabilizer transparently and automatically places skeleton nodes in the middle of the process section. The automatic node position computation is performed by shooting straight rays onto all 4 directions along the coordinate system's main axes, and computing the average distance to the surrounding wall boundaries. This simple but effective method provides a way to rapidly trace main cellular processes, and create fully controlled skeleton representations.

6. Setup and results

Our proposed immersive system is used by neuroscientists for performing real-time creation, proofreading/editing, and exploration of brain cell reconstructions based on medial axis representations. In this section, we first describe our setup (implementation details, and data preparation) then report initial results on five highly detailed full cell reconstructions obtained by serial section electron microscopy [CAK*19].

Implementation details. The immersive system have been developed and deployed using the Unity game engine (version 5.6.3, via C# scripting). For VR, it uses SteamVR and the VRTK software packages [Mur17], which provide smooth immersive system-user interaction as well as cross-hardware setup compatibility. In this way, the same application can be used on other VR setups, e.g., Oculus Rift [DDAM14]. For computing automatic skeletons, and for other preprocessing tasks, we implemented and used C++ applications and Python scripts. In addition, we used Avizo (a commercially available data analysis/visualization software framework) for computing high-quality skeletons. We tested the VR application on a gaming laptop equipped with an Nvidia GTX 1080 8GB GDDR5X GPU, while preprocessing was carried out on a workstation equipped with two CPUs of 10 cores each (see table 2 for additional details).

Machine	OS	Task	Specs
Asus ROG G703G	Windows 10 Pro	Immersive environment	32GB DDR4, Intel Core i9-8950HK 4.8 GHz, Nvidia GTX 1080 8GB GDDR5X, 2X 256GB PCIE SSD + 2TB SSHD FireCuda.
Supermicro	Linux CentOS 7	Data processing and skeleton creation	1TB memory, Intel(R) Xeon(R) Gold 6150 CPU2.70GHz (18 Cores), Nvidia GK104GL Quadro K5000, N/A

Table 2: *Machines used for immersive environment and data preparation.*

Data preparation. For testing purposes, we considered five complex cellular structures reconstructed from a p14 rat somatosensory cortex. We selected different kinds of cells to show different levels of complexity: two neurons, two microglia, and one astrocyte [CAK*19]. The cells were reconstructed from a high-

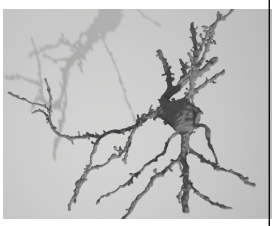
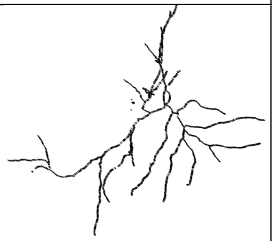
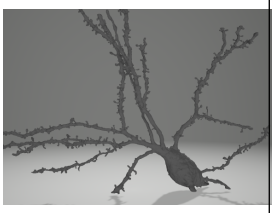
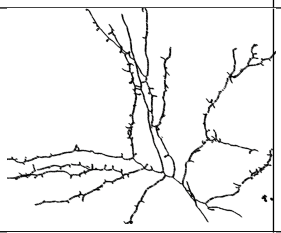
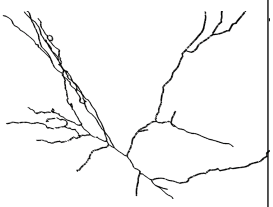
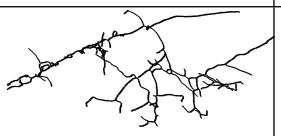
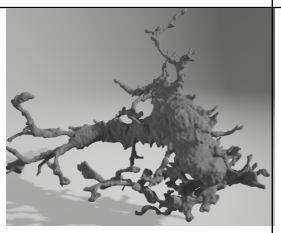
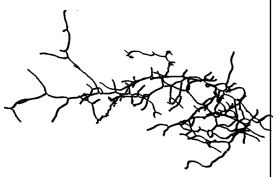
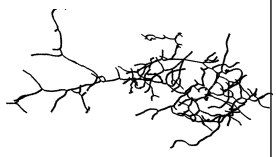
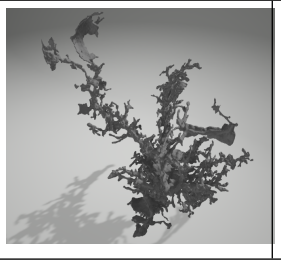
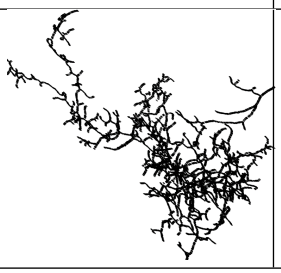
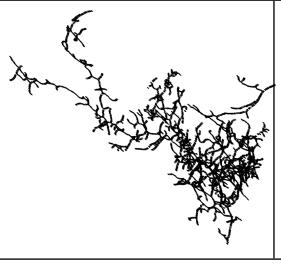
Name	Cell Morphology	Total Vertices	Time mm:ss	MC Skeleton	Nodes Edges Branch	MCS-Proofread	Nodes Edges Branch
Neuron1		49,628	10.00		1569| 1573| 201		1318| 1321| 25
Neuron2		78,215	15.28		1,619| 1,629| 357		1215| 1223| 20
Microglia1		48,015	09.00		1,463| 1,479| 165		1443| 1456| 62
Microglia2		125,532	13.53		2,105| 2,122| 260		2060| 2077| 111
Astrocyte		211,004	25.16		4,055| 4,137| 854		3906| 3983| 296

Table 3: ***Morphologies and Mean Curvature Skeletons (MCS) of 5 biological cells.*** *Cells are computed automatically through [TAOZ12] and proofread and cleaned through our Virtual Reality system. Together with pictorial representations, we report on cell sizes, total times for proofreading and cleaning, and skeleton statistics.*

resolution EM stack with approximated size of 100μm X 100μm X 76.4μm (see Fig. 2 left). The reconstruction process was performed through a semiautomatic process [CCK*18] involving customized components and public domain software like iLastik [SSKH11] and TrakEM2 [CSS*12]. The output of the reconstruction process is a series of high resolution triangular meshes representing the cellular morphologies (see Fig. 2 right). Furthermore, each cell was optimized in a way to be watertight and without non-manifold edges and vertices, and in a way to preserve all important morphological features. To this end, we used public domain mesh processing tools like Blender [Hes07], Meshlab [CCC*08], and Ultralizer, a geometry processing tool contained inside the suite NeuroMorphoVis [AHE*18]. For getting automatic medial axis representations of the considered morphologies, we used the Mean Curvature Skeleton algorithm [TAOZ12], as well as the Centerline Tree module both available in Avizo [SBB*00]. In table 3 we report on the cell morphologies and the associated skeleton representations. Specifically we provide visual representations of the morphologies, together with information about their shapes and sizes in terms of vertex counts, visual representations of automatic skeletons, and skeleton graph statistics (number of nodes, number of edges, and number of branches).

7. Evaluation

A preliminary evaluation of the system was performed by two expert neuroscientists on cells of table 3. Domain scientists were particularly interested in obtaining accurate and clear skeletal representations to be used as descriptors of highly intricate cellular structures. In general, they want to have precise control of medial axis representations, in a way to be able to clearly separate main processes from fine details that have different biological meaning (for example dendritic shafts and spines in neurons). In this sense, most automatic systems provide "dirty" medial axis representations, thus we expected that an interactive tool helping in cleaning skeletons would receive a positive feedback. Moreover, we expected that the immersiveness provided by virtual reality could improve the creation and editing process.

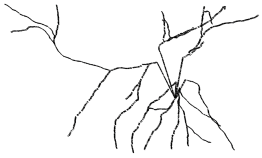
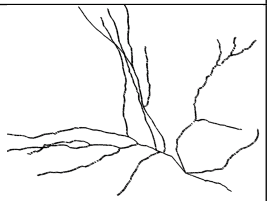
Cell Name	VR Native Skeleton	Run Time mm.ss	Nodes| Edges| Branches
Neuron1		25.13	481 | 480 | 25
Neuron2		30.50	629 | 628 | 20

Table 4: *Statistics on skeletons generated semi-automatically from scratch for Neuron1 and Neuron2 morphologies.*

Skeleton creation from scratch. Neuroscientists used the system for creating skeletons from scratch on two neural morphologies. In table 4, we show statistics about the skeleton creation process. The procedure consisted of exploring the surface models in order to select the main processes, and trace the branches from inside the cells, i.e., similar to an endoscopic navigation/view. Domain scientists felt comfortable in recognizing main processes, e.g., dendrites and spines, in a way to correctly trace the medial axis of interest. Moreover, they felt quite comfortable with the path stabilizer, which reduced the number of input actions on controllers.

Cell Name	Algorithm	Nodes|Edges|Branches
Neuron1	MCS	1,569 | 1,573 | 201
	CLT	7,719 | 7,328 | 361
Neuron2	MCS	1,619 | 1,629 | 357
	CLT	9,655 | 9,530 | 516

Table 5: *Neuron1 and Neuron2 skeleton properties as generated via Mean Curvature Skeleton (MCS) and Centerline Tree (CLT) algorithims.*

Skeleton proofreading/editing. Automatically computed skeletons were examined by domain scientists through the proposed system (see Fig. 6). They used the system for comparing skeletons automatically computed through Mean Curvature Flow (MCS [TAOZ12]), and Centerline Tree (CLT [SBB*00]). They concluded that both the methods considered were able to cover all the morphology features of interest. However, skeletons produced by CLT appeared to be too highly detailed, with a number of wrongly assigned branches as well as disconnected parts. Table 5 shows the difference in the total number of branches, nodes and edges for each algorithm for all five cells. In general, domain scientists found that skeletons produced by MCS algorithm contained a lower number of artifacts. For this reason, in all considered cases, they preferred to perform editing and cleaning on skeletons computed through MCS algorithm. To this end, they carried out a series of checks depending on the type of cell, and on the biological significance of the various features:

- Identify main branches by tagging their nodes as either leaf, end of branch, or internal nodes. The system identifies all node types based on the degree of each one in the graph tree. However, some needs to be adjusted based on the cell's biological features. Using the VR interactive menu, the user points at a node with the VR controller's laser pointer and then clicks on the trigger button to tag it. The node's color material will switch color indicating that it is saved in the system based on the tagging feature. In the case of neurons, the main branches would be all dendrites, excluding any other features e.g., spines.
- In the case of highly-detailed skeletons, one would encounter duplicate nodes and edges, disconnected parts, loops, and out of track skeletonization. Neuroscientists tried to delete all defects through an iterative manual process.
- The soma area should be clear from any branching so neuroscientists "cleaned" these parts by deleting all branches and merging them into one.

In last two columns of table 3, we show the proofreading outputs

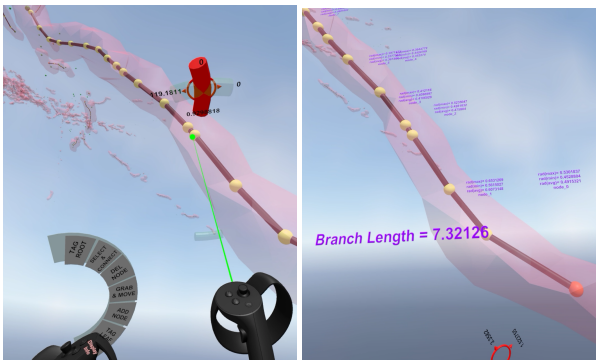


Figure 7: ***Branch-based measurements.*** *Our system performs calculations of measurements on intracellular structures. Left: User points the laser pointer at any node of a branch of interest to display node-relevant measurements. Right: Measurements of a mitochondrion branch are displayed.*

for all the considered cells. In general, domain scientists found the proofreading task comfortable and accurate, and they particularly appreciated the immersiveness of the system for checking features and recognizing defects.

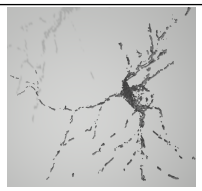
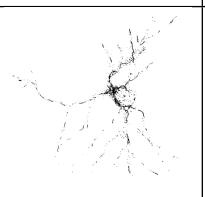
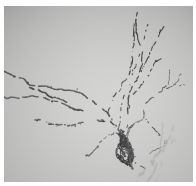
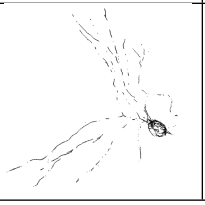
Cell Name	Morphology	MC Skeleton	Nodes| Edges| Branches
Mito Neuron1			2,246| 1,963| 1,396
Mito Neuron2			1,749| 1,656| 811

Table 6: *The intracellular structures of Neuron1 and Neuron2 showing mitochondria morphology, side by side with their skeletons generated via the MCS algorithm.*

Exploration and analysis of branch-based intracellular measurements. One of the significant benefits of having skeleton representations of brain cells is the possibility of computing accurate measurements of morphological features. As a preliminary test, neuroscientists performed analysis of mitochondria, which are intracellular structures within the neural cells Neuron1 and Neuron2 (see Table 6). Since scientists are particularly interested in measuring specific geometric features of organelles, like lengths and radii (maximum, minimum, and average), adequate skeleton representations are needed for performing accurate measures. To this end, our system uses the same functionality equipped in the VR path stabilizer. Users can point at a particular node from a branch of interest, and the system uses the skeleton information for providing the measure of the full length, along with the radius values at each skeletal node contained in that branch. The measured values are shown as text labels in the scene on top of each node and recorded for subsequent statistical analysis (see fig. 7).

Discussion. In general, one of the drawbacks of dealing with an immersive environment on long sessions (15 minutes and more), is the evident symptoms of cybersickness and fatigue. This happened also for our system, and, during all sessions, neuroscientists needed to take breaks every 10 minutes when performing each task. To this end, the system allows for multiple saves across sessions, and the user can retrieve the file anytime and continue where he/she last stopped. As general impression, the system was considered very useful for proofreading/cleaning pre-exported skeletons, but not as much for creating skeletons from scratch. Neuroscientists found the process of creating skeletons from scratch in VR time-consuming. By observing the behavior of users with the system, we could note that several factors contributed to make the creation process time consuming. One factor involves the order of tracing the various branches. Specifically, in some cases, users started tracing from the soma and proceeded towards the tips, while in other cases they made the opposite choice, by starting from the tip of the most extended branch and tracing towards the soma. Another factor was the time needed to correct placement mistakes, since a single mistake requires three actions for being corrected:

- delete the wrong node;
- add a new node;
- select a parent node in the path to re-connect the new node.

We also experienced that another source of error was the path-stabilizer, in cases where the user happened to release a node at a bifurcation spot. Since the path-stabilizer is based on the concept of ray-casting, users needed to take care of correctly keeping the VR controller within the walls of the cellular structure. Issues with the stabilizer were experienced also in cases when the cell's main branch has too many spines within close distance to each other. In such situations, neuroscientists sometimes preferred to disable the stabilizer and operate on a full-manual mode. In general, we noticed that most creation issues were alleviated as users gained experience with the system, and we think that performance should dramatically improve once users repeat the process for many cells, i.e., after further training and experience. From the quality point of view, neuroscientists were satisfied with skeletons generated from scratch in VR, since they appeared well-structured and represented precisely the biological structure of the cells, tailored accordingly to their experience and knowledge about cell morphology. Regarding the proofreading task, domain scientists performed very well in checking and editing all five skeletons. They experienced some problems only with the Astrocyte, which took around 25 minutes to be proofread and edited.

8. Conclusion

We presented an immersive system for creating, proofreading and exploring medial axis representations from highly detailed

brain cellular morphologies reconstructed from serial electron microscopy. The system is currently used by neuroscientists for deriving accurate skeleton representations to be used for classification, measurements, and simulation purposes [KRS*19]. As future work, we plan to carry out a more rigorous user study to evaluate and compare the strengths the proposed system to standard manual tools routinely used in neuroscience domain to trace neural processes [LBA11, SRHE15]. We also plan to use the same controllers with a large-scale monocular display in a way to alleviate unpleasant side-effects like cyber-sickness and fatigue, while still providing the ability to edit the skeleton in an immersive way.

Our subjective preliminary evaluation showed that domain scientists felt particularly comfortable in using the system for proofreading and editing previously computed skeletons while they still consider the process of creating medial axis representations from scratch to be time consuming. For alleviating this problem, we plan to improve the system by considering online collaborative schemes, in a way to distribute the creation process among multiple users, reduce the working time and effort, and at the same time increase the quality of the output representation. Moreover, we plan to integrate visual analytics tools for exploring feature distributions inside morphologies [SLC*18] and tools for performing visual analysis of topological data representations associated to medial axis representations [KDS*18].

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