NeuroVerse: Immersive exploration of 3D ultrastructural brain reconstructions for education and collaborative analysis

Corrado Calì
corrado.cali@unito.it
Department of Neuroscience "Rita Levi Montalcini",
University of Turin, Turin, Italy
Neuroscience Institute Cavalieri Ottolenghi
Orbassano (TO), Italy

Marco Agus
magus@hbku.edu.qa
College of Science and Engineering,
Hamad Bin Khalifa University
Doha, Qatar

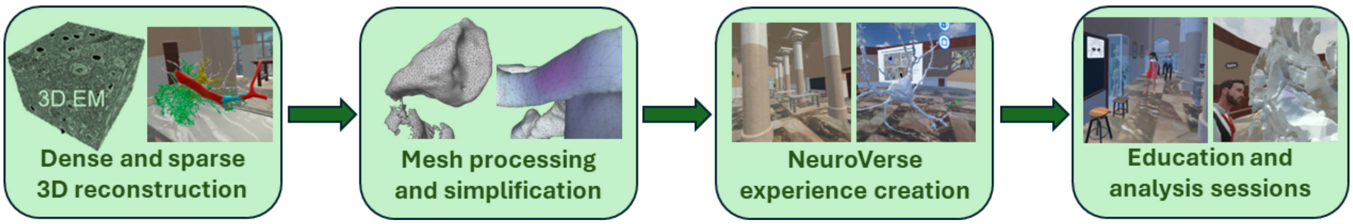

Figure 1: NeuroVerse. We introduce a framework for supporting immersive exploration of 3D nanometric scale reconstructions of brain cellular structures. The pipeline is able to reconstruct 3D mesh models from image stacks acquired through serial section electron microscopy (left), to compose them with absorption signals (center), to process them in a way to allow deployment to a Metaverse environment, and to create immersive experiences targeting neuro-anatomy education and collaborative analysis (right).

ABSTRACT

We introduce NeuroVerse, a framework designed to support the immersive exploration of 3D nanometric-scale reconstructions of structural and ultrastructural neural or glial cellular processes of the central nervous system. Utilizing image stacks acquired through volume electron microscopy, NeuroVerse reconstructs detailed 3D mesh models and integrates absorption signals, enabling deployment within a Metaverse environment. This framework facilitates innovative educational and collaborative analysis experiences, particularly in neuroanatomy and neuroscience. We present a comprehensive methodology, including a pipeline for 3D model creation, segmentation, mesh reconstruction, and heatmap computation, optimized for the Spatial.io ecosystem. Our contributions include the development of a virtual anatomy lab for immersive neuroanatomy education and collaborative sessions focusing on morphology spatial correlation and neuroenergetic absorption models. Preliminary results indicate significant potential for enhancing neuroscience education, improving remote collaboration among scientists, and democratizing access to advanced neuroscientific data and tools.

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CCS CONCEPTS

• **Computing methodologies → Virtual reality**; • **Applied computing → Life and medical sciences**; *Education*; • **Information systems →** *Multimedia streaming*.

KEYWORDS

Ultrastructural analysis, Immersive exploration, Volume Electron Microscopy, Brain reconstructions, Metaverse for education, Metaverse for neuroscience

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1 INTRODUCTION

The vision of the Metaverse as a shared virtual space where multiple users can engage in various daily activities is continually evolving across multiple domains. The primary objective is to enable users to interact remotely using VR devices, facilitating augmented social tasks with the impression of receiving realistic physical inputs [Tukur et al. 2024]. This is more and more possible, thanks to increased computational power of unteathered commercial portable VR headsets. Common examples include multi-user gaming, cyber-physical fashion and musical events, and general social interactions [Tukur et al. 2023]. The need for innovative and engaging educational methods has recently emerged, especially during the pandemic period [Chen et al. 2023]. This is particularly relevant for complex subjects requiring 3D spatial understanding, such as medical anatomy, which has traditionally been taught using physical replicas. Recent efforts have aimed at delivering high-quality experiences for teaching anatomy [Moro 2023].

Simultaneously, the advent of volumetric scanning technologies has revolutionized basic neuroscience research. Serial section microscopes can perform three-dimensional microscale acquisition of brain sections at a nanometric level, enabling visual and quantitative analysis of cellular, and sometimes molecular, structures [Peddie et al. 2022]. Technologies for semiautomatic and automatic 3D reconstruction of brain structures have been developed, facilitating high-quality ultrastructural analysis [Calì et al. 2019; Calì 2024]. Various standalone frameworks have been proposed for immersive exploration and quantitative tasks, ranging from understanding neuroenergetic mechanisms [Agus et al. 2019] to tracing branched structures [Boges et al. 2020b] and neural connections [McDonald et al. 2020].

However, these technologies have not yet been integrated into shared Metaverse spaces, operating instead within isolated virtual environments lacking sharing and remote interaction capabilities. This paper addresses this gap by proposing a processing pipeline to deploy 3D neuroscience investigation experiences in a shared Metaverse space. Specifically, we introduce NeuroVerse, a framework for supporting immersive exploration of 3D nanometric scale reconstructions of brain cellular structures (see Fig. 1). Our framework is able:

- to segment and reconstruct 3D mesh models from image stacks acquired through serial section electron microscopy;
- to map absorption signals (GLAM) on neurites as mesh textures and to process them in a way to allow deployment to a Metaverse environment. To this end we use a radiance-based model integrating the contribution of the glycogen granules in form of heatmap [Agus et al. 2018];
- to create engaging immersive experiences targeting neuro anatomy education and collaborative analysis for neuroscientists.

Contributions. In summary, we provide the following contributions:

- A pipeline for the creation of 3D neuroscience models designed for spatial analysis and customized for immersive and collaborative Metaverse exploration.
- A test case showcasing the use of highly detailed reconstructed models for neuroanatomy teaching sessions within an immersive shared virtual anatomy room.
- A test case describing collaborative analysis sessions focusing on morphology spatial correlation analysis and the assessment of neuroenergetic absorption models within a shared Metaverse environment.

To our knowledge, this work represents one of the first initiatives to introduce immersive neuroscience exploration into the Metaverse. We believe that this technology will significantly enhance and democratize neuroscience education, improve collaboration and information exchange among remote scientists, and facilitate the sharing of analysis data between research groups.

2 RELATED WORK

Our work allows multiuser access of an interactive Metaverse for medical and neuroanatomy education, as well as the development of immersive visualization environments for supporting neuroscience investigations. We don't aim to provide an extensive literature review of these topics, but we suggest interested readers to consider recent surveys about modern methods for teaching digital anatomy [Adnan and Xiao 2023; Iwanaga et al. 2023; Moro 2023], and about visual analysis of neuroscience data [Beyer et al. 2022]. In the following we discuss the literature most closely related to our research.

Metaverse in medical education. Since the advent of Metaverse, education experts and practitioners investigated sophisticated ways to deliver educational content for topics involving complex 3D spatial interaction, especially for the medical field [Moro 2023]. More recently, collaborative spaces have been proposed for delivering engaging content in a way to simulate authentic in-the-field experience for practicing students [Wu et al. 2022]. Examples of these efforts include:

- methods for teaching anatomy basics in immersive environments [Nakai et al. 2022],
- environments for practicing virtual dissections with the goal of improving motor skills during laparoscopic tasks [Ebina et al. 2021],
- interactive virtual environments for microsurgery anatomy education [Gonzalez-Romo et al. 2023],
- creation of remote diagnosis sessions with virtual patients targeting diabetes conditions [Takahashi et al. 2021],
- development of specific content for teaching complex 3D features related to ostiomeatal complex for dentistry applications [Iwanaga et al. 2022],
- an immersive environment for teaching neuroanatomy basics on 3D photogrammetry reconstructions of brains [Aridan et al. 2024].

The main goal of these efforts is to enhance the visualization of complex 3D morphologies to improve the understanding of their anatomy, but also to boost the interaction between teacher and students and allow for simultaneous access of multiple teaching content [Nakai et al. 2022]. Despite the promising results of the described initiatives, still various challenges need to be addressed, mostly related to the assessment of immersive technologies [Chytas et al. 2023], and the typical risks of metaverse applications, related to security, and privacy aspects [Tukur et al. 2023], that are more pressing when it comes to medical content. On the other side, there is still lacking of specific content for teaching specific neuro-anatomy topics involving small scale brain features, like the basic mechanisms of neural connections [Ramaswamy 2024] or the role of glia in brain metabolism [J. Magistretti and Allaman 2022]. To date, several high-resolution database are made available from the vEM community, for data mining or visualization [Shapson-Coe et al. 2024; Turner et al. 2022], but is not immersive, or oriented on learning and teaching. In this work, we fill this gap by proposing a Metaverse-enabled immersive environment for teaching neuroanatomy for medical students by using nanometric reconstructions of brain cellular structures.

Immersive Environments for Neuroscience investigation. Since the recent advent of methods for 3D scanning of brain samples involving serial section electron microscopy [Peddie et al. 2022], neuroscientists have the chance to analyze and quantify structural and ultrastructural features of neurons and glial cells and their processes at nanometric scale [Calì et al. 2019]. Very recently, various pipelines have been developed for performing reconstruction of neural connections [Abdellah et al. 2023; AU Calì et al. 2019; Boges et al. 2020a; Campbell et al. 2024; Coggan et al. 2018], and these efforts paved the way for the development of immersive environments for supporting visual exploration, collaborative discussion and quantitative analysis of 3D data. The main examples of these efforts include:

- an immersive environment based on CAVE for performing quantitative analysis of glycogen distributions on hippocampal glial and neural reconstructions from a rat brain samples [Calì et al. 2016];
- immersive environments for CAVE and HMD displays for visual analysis of energy absorption mechanisms based on a glycogen radiance model on hippocampal rat samples and on somatosensory cortex samples for understanding effects of aging [Agus et al. 2018];
- a virtual environment for performing analysis, simplification, and correction of branched representations of neural and glial structures [Boges et al. 2020b];
- virtual environments for tracing neurons [Usher et al. 2017] with the aid of topological elements based on Morse-Smale Complex [McDonald et al. 2020] and with the support of deep learning technologies [Hou et al. 2023].

Despite these efforts, after the emergence of Metaverse technologies, there is lack of initiatives for deploying immersive experience for supporting neuroscience investigations. In this work, we propose a pipeline for creating 3D neuroscience content and uploading it in a Metaverse space for supporting collaborative exploration of nanometric 3D reconstructions enriched with absorption signals [Agus et al. 2018].

3 METHODOLOGY

We developed a processing pipeline for deploying meaningful and engaging immersive experiences in the Metaverse, by considering the main results of previous ultrastructural neuroscience investigations. Specifically a project for glycogen analysis on rat hippocampal neuropil reconstruction [Calì et al. 2016], a project for the analysis of energy absorption from neurites in hippocampus and somatosensory cortex [Agus et al. 2018, 2019] and a sparse reconstruction of brain cells in parenchyma from a juvenile rat sample [Calì et al. 2019]. The processing pipeline is depicted in Fig. 2 and it is composed by the following steps: ultrastructural reconstruction, mesh processing and signal computation, and model deployment to the Spatial.io immersive ecosystem. In the following we describe the main components of the process for creating 3D brain nanometric reconstruction for the Metaverse space.

Ultrastructural reconstruction. The models and scenes considered in this work are obtained through processing of 3D image stacks obtained with serial section electron microscopy, that is able to produce images with pixel resolution of 5 nanometers and slices in a range of 5 to 50 nanometer, depending on the sectioning technique [Calì et al. 2018]. These image volumes contain 16-bit density images that need to be labelled to obtain dense or sparse segmentation of the structures of interest. Labelling can be obtained through semiautomatic methods involving image processing software [Berg et al. 2019; Holst et al. 2016; Ma and Wang 2023], and it is done in a way to obtain voxel connected components that can be visualized through volume visualization methods [Agus et al. 2022, 2019], or can be processed to create surface models for supporting quantitative analysis and visual exploration [Boges et al. 2020b]. In our case, we consider a semiautomatic pipeline for labelling image stacks [Coggan et al. 2018], followed by an effective tessellation method based on a voxelization-based remeshing engine that can reconstruct topologically accurate, adaptively optimized, and watertight surface manifolds [Abdellah et al. 2023]. On top of the 3D reconstructed models, we compute absorption signals based on radiance simulation applied to glycogen granules reconstructed from the stacks and we apply these signals in form of heat maps through texturing [Agus et al. 2018]. The output of this processing pipeline is a series of 3D models representing different brain cells, with color mapping related to the kind of cellular structures, or with associated signals and point of interests associated to specific neuroenergetic patterns (in form of colored spheres).

Spatial ecosystem. Between the various ecosystems for developing shared immersive environments, we chose Spatial.io for its flexibility in managing 3D content and for its easy integration with VR headsets and Web3D standards. Specifically, Spatial.io Metaverse [Sriworapong et al. 2022] ecosystem offers a robust platform for creating, sharing, and experiencing 3D content. Users can seamlessly upload and interact with various 3D models, environments, and assets, fostering an immersive and collaborative virtual space. Key features of the Spatial.io ecosystem include:

- **3D Content Creation and Import:** Users can easily create and import 3D models from popular design tools, allowing for the integration of detailed and complex objects into the Metaverse.
- **Interactive Environments:** The platform supports the development of interactive 3D environments, enabling users to navigate and explore virtual spaces in real-time.
- **Collaboration Tools:** Spatial.io provides various tools for real-time collaboration, including voice and video communication, shared whiteboards, and interactive elements, facilitating seamless teamwork and social interactions.
- **Customization Options:** Users can personalize their avatars, virtual spaces, and objects, enhancing the overall immersive experience and reflecting individual preferences.
- **Cross-Platform Accessibility:** The ecosystem is accessible across multiple devices, including VR headsets, desktops, and mobile devices, ensuring a wide reach and flexibility for users.
- **Event Hosting and Participation:** Spatial.io allows for the hosting and participation in virtual events, such as meetings, conferences, and social gatherings, providing a versatile platform for various activities.

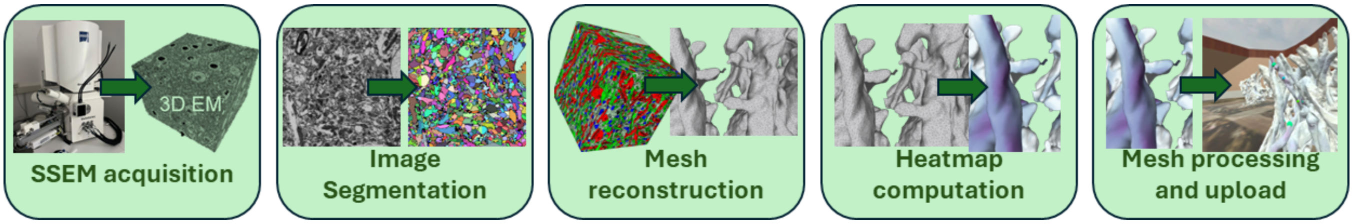

Figure 2: Processing pipeline: in order to create 3D models for immersive visual analysis, we designed a processing pipeline from 3D microscopy image acquisition up to upload to Spatial environment, passing through image segmentation, mesh reconstruction, and heatmap computation. The final output are 3D models in gltf format.

Model deployment. Spatial.io accepts the most popular file formats for 3D assets. For flexibility, and in a way to conform to the best practice for streaming and web sharing, we decided to design a pipeline for creating gltf 3D assets [Lentz et al. 2021]. For doing that, we used popular open source software for 3D processing and we performed the following operations:

- **Mesh Import and Conversion:** we used alternatively Blender [Villar 2021], MeshLab [Cignoni et al. 2008], and online conversion tools to pass from OBJ file format (output of Ultraliser), to Collada DAE (used as interchange format), up to GLB/gltf (used as final format for deployment to Spatial.io);
- **Color Baking:** we used Meshlab to convert texture information to vertex colors to avoid problems related to deployment of models with textures (to this end, we used the Texture to Vertex Color filter);
- **Mesh Merge and Simplification:** we used Meshlab Layer Flattening filter for merging various meshes containing different objects like neurites or absorption spherical peaks. After that we applied a mesh simplification fiiter based on quadrics edge collapse [Garland and Heckbert 1997] to reduce the size of the output models to the number of vertices recommended by Spatial.io. After this processing step, we exported the models in DAE format.
- **Model Export:** we used an online converter (https://convert3d.org/convert) to convert DAE mesh models to GLB files ready to be uploaded in Spatial.io.

4 RESULTS

In the following, we describe the data used in this pilot study, and we report about the education and analysis test cases carried out with the 3D brain cell reconstructions. The Spatial Environment as well as the data used for our test cases can be found in the following URL: https://www.spatial.io/s/Univerista_Anatomia_v2-5-66487bb2140c76ac5e530c9d. Most of the images are extracted from recorded from live sessions in Spatial (the supplementary video in https://bit.ly/4bN1nus contains the main highlights of the analysis sessions).

4.1 Data description

In this project, we considered a virtual environment replicating the dissection lab of the human anatomy institute in the University of Turin, and various 3D models obtained by processing data acquired from previous ultrastructural analysis projects.

Figure 3: Spatial environment: professional 3D artists used Blender modeling software and Unity for creating immersive environments for lectures and collaborative sessions, by modeling a realistic replica of a portion of an European historical anatomy lab.

Virtual Environment. The main shared environment has been developed in Blender and Unity by professional 3D artists and it represents a realistic blueprint of a portion of the historic Anatomy institute in the University of Turin. The virtual environment is composed by two rooms connected together:

- a dissection room, realistic replica of the space where anatomy labs are taken and students can assist and practice dissections;
- a semicircular amphitheater for lectures and seminars, where scientists can perform collaborative sessions and virtual analysis of data.

Rat hippocampus. The 3D models are processed from an EM stack extracted from juvenile rat hippocampus, and they represent dense reconstructions of astrocytic processes ensheating neurites (dendrites, axons, spines, boutons), relevant inner structures (endoplasmatic reticulum and mitochondria) and glycogen granules [Calì et al. 2016]. The models are enriched with energy heatmap signals representing the probability density function of glycogen energy absorption on the various cellular structures [Agus et al. 2018]. These models are particularly valuable for performing exploratory analysis, to understand the 3D organization and layout of the various cellular structures and to generate hypothesis and investigation plans.

Rat parenchyma. The 3D models are processed from a big stack extracted from the parenchyma of a juvenile rat, and they represent sparse reconstructions of full cells including neurons, astrocytes, microglia, pericyte, and the vascular vessel [Calì et al. 2019]. These models are particularly relevant for a morphology perspective since they can provide accurate measurements and parameters that can be used for simulation purposes [Shichkova et al. 2021].

Figure 4: Immersive neuroscience lecture: An anatomy professor delivered an immersive lecture on ultrastructural analysis of brain cellular structures. Four students participated using Meta Quest 3 headsets, allowing them to attend the lecture within the Metaverse.

4.2 Education Session

To explore the potential of immersive learning in neuroscience, we conducted an experimental lecture in the Metaverse using the Spatial environment, which replicates an anatomy lab. This setup, combined with processed 3D models, PowerPoint presentations, and microscope image examples, created a comprehensive educational experience. The class consisted of four students enrolled in a Master in Biotechonology for Neuroscience,specifically in a Computer Vision course. Each student used a Meta Quest 3 head-mounted device to fully immerse themselves in the virtual classroom (see Fig. 4). The lecture focused on analyzing serial section microscopy images and identifying various micro and nanoscale cellular features in rodent brain samples. The professor used the 3D models extracted from rat hippocampus and parenchyma to illustrate key concepts (see Fig. 5).

During the lecture, the teacher presented the following brain cell structures in detail (see Figs. 5 and 6):

- The main neural processes, such as dendrites, spines, and boutons.
- Details about synaptic formation and the connection between spines and boutons.
- Highlights of post-synaptic densities, specifically annotated using the scribble tool in Spatial.
- Details about the role of vesicles in storing and releasing neurotransmitters.
- The correspondence between 3D reconstructions of neurites and labeled Electron Microscopy images, specifically the synapse and bouton (see Fig. 7).
- A showcase of the main morphological features of astrocyte cells.

The immersive environment provided a detailed and interactive way to teach neuroanatomy, since the instructor was able to deliver neuroanatomy basics using 3D nanometric reconstructed models in a virtual environment that perfectly reconstructed a dissection room and an amphitheater for anatomy lectures. He was able to give detailed descriptions of micro and nanoscopic cellular features in 3D with supporting figures from videos and presentations and he was able to highlight strategic details with annotation scribbles. Importantly, master students with a neuroscience career plan often ignore size, shape and relationship of the most basic components of the neuropil; all the partecipants percieved this experience as an added value to their knowledge.

4.3 Collaborative analysis

Two connectomics experts (also authoring the paper) performed various analysis sessions on the models reconstructed from data coming from previous projects:

- glia reconstructions from a hippocampus rat sample [Calì et al. 2016];
- morphology reconstructions of entire cells from a parenchyma sample [Calì et al. 2019];
- glycogen absorption models on neurites from a hippocampus rat sample [Agus et al. 2018].

In the following we describe the three analysis sessions. Additional material can be found in the supplementary video in https://bit.ly/4bN1nus.

Hippocampal glia processes. Two neuroscientists performed a collaborative visual analysis of the main process composing the glia reconstruction [Calì et al. 2016], by checking the three dimensional morphologies, and by getting inside the main processes to understand the distribution of the glycogen granules (represented as small spheres), and the various organelles, explicitly endoplasmatic reticulum (in yellow) and mitochondria (in purple) (see Fig. 8). The domain experts performed discussion on top of annotations highlighting the specific features of the models, and made hypothesis about correlation between organelles and glycogen granules, by noticing for the first time the following patterns :

- the persistent proximity of endoplasmatic reticulum and glycogen clusters inside the main astrocytic processes (Fig. 8 top right);
- the particular shape of endoplasmatic reticulum in some areas, embracing and surrounding glycogen granules (Fig, 8 bottom left);
- the proximity of endoplasmatic reticulum to mitochondria and the characteristic shape to suggest a close interaction (Fig. 8 bottom right).

Figure 5: Education test case: The 3D reconstructed models, along with additional material (extreme left), were used to explain neuroanatomy basics, including the morphology of main neurites (center left), the correspondence between 3D reconstructed neurites (center right), and the morphology of astrocytes (extreme right).

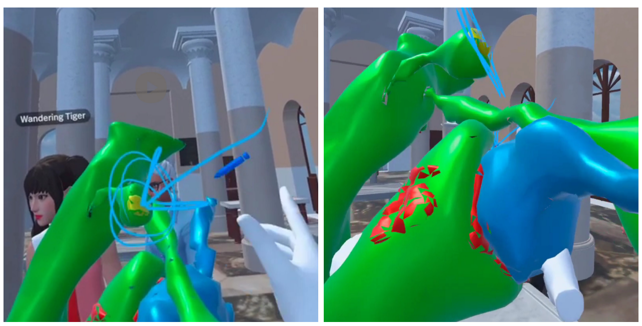

Figure 6: Neurite details: the instructor created annotations highlighting the post-synaptic density (in yellow, left) and detailed the synaptic connection, showing the bouton (in blue), spine (in green), and vesicles involved in neurotransmission (in red, right).

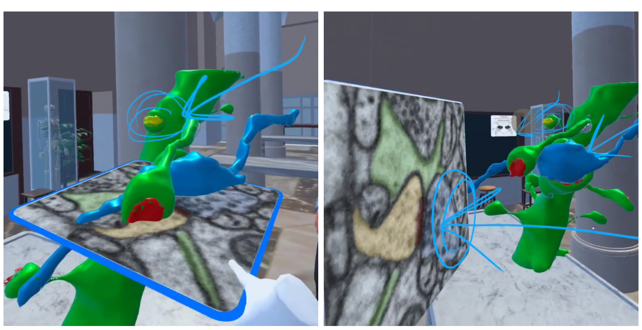

Figure 7: EM mapping: the instructor highlighted the correspondence between specific neurite reconstructions and their corresponding portions in Electron Microscopy images. On the left, the spine and post-synaptic density, and on the right, the EM mapping for the bouton.

In the discussion, we provide some insights about the consequence of this analysis for formulating hypothesis about the role of organelles in glycogen storage, transport, and conversion to lactate

Figure 8: Hippocampus glia analysis: two neuroscientists performed a collaborative analysis of glia processes (top left) reconstructed from a rat hippocampus, where they investigated the spatial correlation between glycogen clusters and endoplasmatic reticulum (top right and bottom left), and between ER and mitochondria (bottom right).

for energy supply [Veloz Castillo et al. 2021].

Parenchyma whole cells. Two domain experts performed an analysis of sparse cell reconstructions extracted from a rat parenchyma sample, representing a neuron, two glia(one astrocyte and one microglia), a vascular cell, and a pericyte [Calì et al. 2019](see Fig. 9). Specifically, they were interested in performing a visual analysis of the neuron and astrocyte and their connection with respect to the rest of the complex, specifically:

Figure 9: Full morphologies visual exploration: two neuroscientists performed a collaborative visual analysis of a sparse Neural-Glia-Vascular ensemble extracted from a rat parenchyma sample (extreme left), to investigate the biforcation of main neural processes (center left), and the main morphology features of the astrocyte, including the perivascular processes (center right), and the inner mitochondria (extreme right).

- the main branching of the neuron, and the peculiar derivation of axon from one of the main dendritic branches (axons are recognizable since they do not contain spines) (Fig. 9 top right);
- the morphology of astrocytes and the position of soma with respect to the other processes;
- the distribution of mitochondria inside the main processes of astrocytes (Fig. 9 bottom right);
- the presence of one or more perivascular processes in the astrocytes for glucose delivery and to form the blood-brain-barrier (Fig. 9 bottom left).

In this case, experts confirmed most of the insights already achieved in previous studies [Cali et al. 2019].

Hippocampal glycogen absorption maps. Two domain experts and two Ph.D. students performed an analysis sessions for exploring dense neurite and glia models extracted from a rat hippocampus sample, and enriched with heat maps and color codes representing absorption patterns related to glycogen proximity [Agus et al. 2018]. The main goals of these sessions were to confirm initial hypothesis about absorption and role of glycogen to neurites formation, and specifically:

- to compare absorption patterns between dendrites, the various kind of axons, and boutons, and formulate hypothesis about memory formation and learning (see Fig. 10 top);
- to relate the main accumulation of glycogen in specific astrocytic processes to the main absorption patterns in neurites (see Fig. 10 bottom).

After collaborative discussion, two absorption hypothesis were formulated for the dendrite with highest absorption patterns and not exhibiting spines: i) it was at the moment inactive and the glycogen was stored for future usage, ii) the dendrite was focus of intense action related to the simultaneous formation of various spines and synapses associated to a learning process.

4.4 Discussion and Limitations

Preliminary Qualitative Assessment. The integration of 3D detailed models and immersive technology provided a highly engaging and effective experience, allowing students and neuroscientists to gain a deeper understanding of complex neuroanatomical structures and processes. In the following, we enumerate the main qualitative insights that we extracted from the explorative sessions:

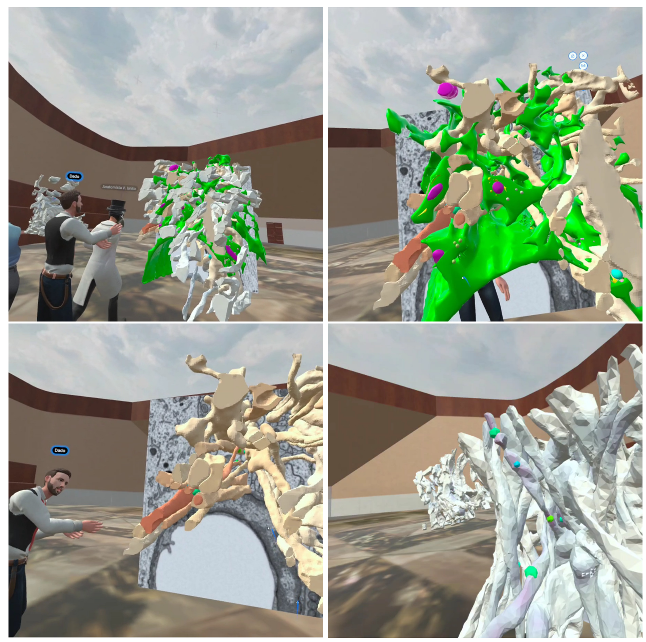

Figure 10: Hippocampus absorption analysis: four neuroscientists performed a collaborative visual analysis of dense models extracted from a rat hippocampus, to investigate absorption patterns related to glycogen energy on neurites (top left), and correlate them to astrocyte processes (center left), the EM image stacks (top right), in a way to speculate about main neuroenergetics mechanisms (bottom left and right).

- **Level of involvement and satisfaction of students:** The use of immersive VR technology significantly increased student engagement. The ability to interact with 3D models in a virtual space allowed students to explore brain structures in ways that traditional 2D images and physical models cannot offer. Feedback from the students indicated a high level of satisfaction, with many appreciating the hands-on approach to learning complex neuroanatomy.

- **Further insights through virtual collaboration sessions:** Virtual collaboration sessions provided a platform for students and scientists to discuss their observations and hypotheses in real-time, enhancing their understanding through peer learning. These sessions also allowed instructors to provide immediate feedback and address misconceptions, fostering a collaborative learning environment.
- **Future assessment for checking how valuable this technology is for education:** While initial feedback is positive, it is essential to conduct more formal assessments to evaluate the long-term benefits of using immersive technology in education. Future studies should measure learning outcomes, and overall performance to quantify the technology's impact.
- **Surveys, questionnaires, performance assessment with separate groups:** To gain comprehensive insights, we propose using surveys and questionnaires to gather detailed feedback from students about their experiences. Additionally, performance assessments comparing groups using traditional learning methods versus those using immersive VR technology will help in understanding its effectiveness.
- **Scientists may speculate about actions happening at the ultrastructural level, and plan additional experiments to test their hypotheses:** The detailed 3D models serve as excellent tools for scientists to brainstorm and hypothesize about cellular mechanisms and interactions. This can lead to the formulation of new research questions and the planning of experiments to test these hypotheses, potentially advancing the field of neuroscience. As matter of example, the neuroscientists highlighted a consistent proximity in the main astrocytic processes between glycogen clusters and endoplasmatic reticulum, suggesting a possible connection and interaction between these organelles. While recently it has been showcased that in the liver the newly formed glycogen is primarily found in ER-rich regions and remains associated with the latter during glycogen deposition and depletion [Mandl 2023], there is currently no evidence of similar mechanisms happening in the astrocytes [Öztürk et al. 2020], apart of glycogen pumping Ca^{2+} into ER [Dienel 2019].

Limitations. Despite the promising initial results, the framework and the pipeline still has main limitations that need to be addressed in future developments:

- **Volume rendering and interactive transparency for highlighting internal structures:** One limitation is the current inability to effectively render volumes with interactive transparency, which is crucial for visualizing overlapping internal structures. This limitation can hinder the comprehensive analysis of densely packed cellular environments, and the glycogen spatial distribution.
- **Automatic registration of 3D models part of the same scene, currently performed manually through markers:** The process of registering different parts of 3D models to form a complete scene is still manual, involving the use of markers. This can be time-consuming and prone to human error. Automating this process would increase efficiency and accuracy.
- **Additional interactive tools for supporting slicing the 3D image stacks, interactive visibility, and change of visual attributes:** The current system lacks advanced interactive tools that allow users to slice through 3D image stacks and dynamically adjust visibility and visual attributes of different structures. These features are essential for detailed analysis and understanding of complex anatomical relationships.
- **Focus on microscale reconstructions:** the proposed framework targets 3D reconstructions from nanoscale imaging. However, recent advances in mesoscopic imaging technologies based on light microscopy [Choi et al. 2024] enabled sophisticated 3D investigations related to atlas-based registration for creating functional and connectivity maps of whole brains [Wang et al. 2020] or for performing 3D reconstruction of large groups of connected neurons in different cortex areas [Gao et al. 2023; Qiu et al. 2024]. It would be of particularly interest for the neuroscience community to develop pipelines able to integrate multi-scale and multi-modal data seamlessly in the Metaverse to support immersive examination at various levels [Manubens-Gil et al. 2023]. We plan to investigate this challenging research direction in future.

Overall, while the initial implementation of NeuroVerse shows great promise in enhancing neuroscience education and collaborative research, addressing these limitations will be crucial for its continued development and broader adoption.

5 CONCLUSIONS

We presented NeuroVerse, a framework for creating 3D immersive experiences for neuroscience education and collaborative scientific analysis. NeuroVerse enables multiple users to engage in real-time exploration and discussion of complex 3D brain reconstructions, in a way to foster a deeper understanding of neuroanatomical structures and to enhance the overall educational experience. At the educational level, the preliminary sessions showcased that the use of immersive VR technology is highly beneficial. Students demonstrated increased engagement and a better grasp of complex 3D patterns and their associations with electron microscopy images. This method of teaching not only makes learning more interactive and engaging but also helps students visualize and understand intricate cellular structures that are difficult to comprehend through traditional methods. In addition to educational benefits, NeuroVerse facilitates improved communication and information exchange between scientists. The platform's ability to support remote brainstorming sessions allows researchers from different geographical locations to collaborate effectively, share insights, and develop new hypotheses. This capability is particularly valuable in today's global research environment, where collaboration and data sharing are essential for scientific progress. Furthermore, NeuroVerse democratizes access to advanced neuroscientific data and tools, making it easier for researchers and educators to share and highlight complex features in 3D processes. By providing an accessible platform for data exchange, NeuroVerse promotes inclusivity and the widespread dissemination of knowledge, which is crucial for the advancement of the field. Looking ahead, future work will focus on assessing the educational improvements brought about by NeuroVerse through controlled experiments. These studies will provide quantitative data on the framework's impact on learning outcomes. Additionally, efforts will be made to integrate more models derived from automatic reconstruction processes [Schmidt et al. 2024; Svara et al. 2022; Xu et al. 2024] and to integrate 3D reconstructions derived from mesoscale light microscopy imaging [Choi et al. 2024], further enhancing the platform's capabilities.

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