Volume xx (200y), Number z, pp. 1–13

ViMO: Visual Analysis of Neuronal Connectivity Motifs

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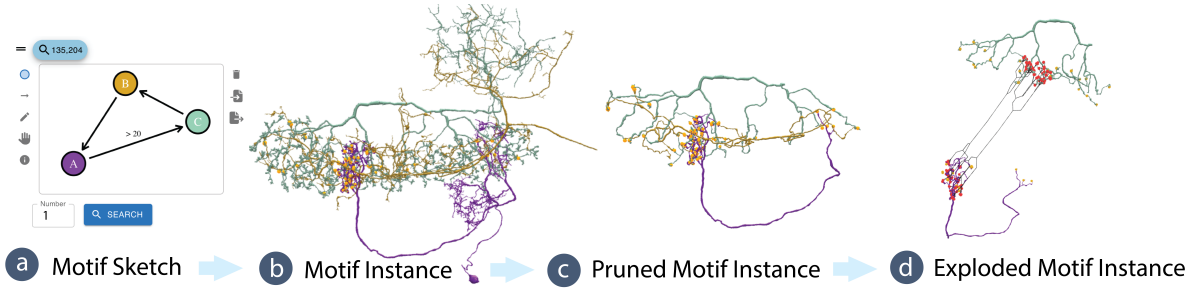

Figure 1: ***Visual Motif Analysis.*** *In* Vimo*, neuroscientists (a) sketch neuronal connectivity motifs and query large connectomics networks for instances of that motif. (b) Neurons forming a motif instance are visualized in 3D.* Vimo *emphasizes the relationship between the sketched motif and the neurons' connectivity using a continuous focus&context approach (c,d). First,* Vimo *prunes unrelated neuron branches to the motif (c). Next, users can further explore the connectivity between neurons in an exploded view that untangles complex neuron morphologies and use hierarchical synapse clustering and bundling to highlight connections (d). Data: FlyEM Hemibrain [SXJ*20]*

Abstract

Recent advances in high-resolution connectomics provide researchers access to accurate reconstructions of vast neuronal circuits and brain networks for the first time. Neuroscientists anticipate analyzing these networks to gain a better understanding of information processing in the brain. In particular, scientists are interested in identifying specific network motifs, i.e., repeating subgraphs of the larger brain network that are believed to be neuronal building blocks. To analyze these motifs, it is crucial to review instances of a motif in the brain network and then map the graph structure to the detailed 3D reconstructions of the involved neurons and synapses. We present Vimo*, an interactive visual approach to analyze neuronal motifs and motif chains in large brain networks. Experts can sketch network motifs intuitively in a visual interface and specify structural properties of the involved neurons and synapses to query large connectomics datasets. Motif instances (MIs) can be explored in high-resolution 3D renderings of the involved neurons and synapses. To reduce visual clutter and simplify the analysis of MIs, we designed a continuous focus&context metaphor inspired by continuous visual abstractions [MAAB*18] that allows the user to transition from the highly-detailed rendering of the anatomical structure to views that emphasize the underlying motif structure and synaptic connectivity. Furthermore,* Vimo *supports the identification of motif chains where a motif is used repeatedly to form a longer synaptic chain. We evaluate* Vimo *in a user study with seven domain experts and an in-depth case study on motifs in the central complex (CX) of the fruit fly brain.*

CCS Concepts

• ***Human-Centered Computing*** → *Visual motif analysis, Focus&Context, Scientific visualization, Neuroscience;*

1. Introduction

Recent developments in high-throughput electron microscopy have allowed large-scale brain mapping at the level of individual synapses, i.e., Connectomics. These new datasets of brain tissue, along with advances in automated segmentation methods, enable scientists to accurately reconstruct 3D wiring diagrams of the biological neural networks. For example, the new *H01* dataset [SCJB*21] captures a cubic millimeter of human brain tissue containing about 57,000 segmented neurons. Once the data is fully proofread, analyzing its neuronal connectivity is the key to understanding how the brain computes.

However, synapse-level connectivity analysis of large brain networks is still underexplored. The reason for that is three-fold: First, only a few datasets exist that are large enough to contain thou-

sands of complete neurons at an image resolution that resolves individual synapses. Previous approaches mainly focused on small volumes and truncated networks [AABS*14] or used heuristics to estimate synaptic connections in lower-resolution data [SBS*13]. Second, large graphs are difficult to analyze, especially for non-experts. Finding motifs in a large graph is computationally expensive, and most previous motif analysis tools require programming experience. Third, neurons and synapses have a dual representation in a network graph compared to the original imaging data. In a network graph, neurons are nodes, and synapses are edges. In brain tissue, however, neurons are long, tree-like structures, while synapses are small surface structures. Few tools can display or analyze large connectivity graphs combined with their underlying imaging data.

One approach for extracting information from large connectivity graphs is searching for common motifs. A graph motif is a recurring subgraph of a larger graph, such as a feedback loop between neurons. Using motif analysis, neuroscientists hope to gain insights into the underlying biological principles of the brain [SK04, GJC22]. For example, researchers have recently identified network motifs in the brain of a fruit fly that are responsible for context-dependent action selection [HHF*21]. However, neuroscientists typically need to combine motif search with an in-depth analysis of motif instances (MIs), which are specific neurons that form a motif. They want to analyze not just abstract connectivity graphs but also explore the anatomy of neurons and understand how neurons in an MI interact spatially. Ideally, a spatial view would allow them to identify the parts of a neuron actively involved in a motif or spatial synapse clusters. However, the structure of neurons is so intricate and complex that even for small motifs, a 3D view of several neurons quickly suffers from occlusion and visual clutter. Therefore, new methods are needed to explore the 3D nature of neuronal motifs without overwhelming the user.

In this design study, we present *Vimo*, a novel visualization and analysis tool to explore neuronal motifs and motif chains in large brain networks. Our work makes three main contributions. First, the design of an interactive and intuitive *motif sketching and querying interface*. Scientists can draw nodes and edges in a sketching panel to query for their desired motif without using standard graph queries or programming. Our query approach leverages biological constraints to reduce computational complexity, scales to large brain networks, and gives interactive feedback on the relevance of the current motif. Second, we propose a *focus&context scheme for analyzing motif instances* in high-resolution imaging data to facilitate the user's understanding of the detailed neuron connectivity. We do so by de-emphasizing non-motif-relevant parts of the neurons (context) in order to focus on the motif-relevant aspects. Importantly, we do not simplify or change neuron morphology. Instead, we gradually shift the focus by using neuron pruning, exploded views, and hierarchical synapse clustering (see Fig. 1). Third, the integration of our motif query interface with our focus&context scheme for MI analysis and its extension to analyze *motif-based synaptic chains* into an open-source application. We developed *Vimo* following the design study methodology [SMM12] in close collaboration with domain scientists in connectomics. We detail our goal and task analysis, report on design decisions, and evaluate *Vimo* in a user study with expert users and a case study of external ring neurons in the fly brain.

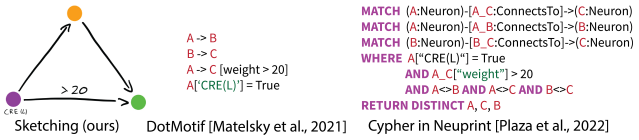

Figure 2: ***Different motif queries.*** *Our visual sketching approach (left), high-level domain-specific language DotMotif [MRJ*21] (middle), and graph query language Cypher [FGG*18] (right).*

2. Related Work

Connectomics and Motif Analysis. Motif analysis plays a central role in connectomic analysis [HHF*21, SBS*21b, UHM*22, GJC22]. Hence, many computational approaches for studying connectivity motifs in connectomes have been presented over the past years. Matejek et al. [MWC*22] present an optimized and parallelized subgraph enumeration algorithm to count the occurrences of motifs in large brain networks. *DotMotif* [MRJ*21] is a domain-specific language to write motif queries and compiles to common python tools and the *Cypher* graph query language [FGG*18]. *Vimo* builds on *DotMotif*, but offers a visual non-programming interface to create motif queries (see Fig. 2).

Visualization for Connectomics. Visual analysis approaches have been used in many subareas of connectomics, including interactive proofreading [GWB*21, DMM*22], volume exploration [BAAK*13], and neighborhood- [TCG*22] and morphology analysis [MAAB*18, CLL*21]. Beyer et al. [BTB*22] provide a comprehensive survey of visualization methods in connectomics. Most neural connectivity visualizations use node-link diagrams [SCHT09, BAAK*13]. Recently, Ganglberger et al. [GWW*22] proposed a 2D node-link layout that preserves the spatial context of 3D brain networks. However, their work focuses on macroscale brain parcellations and thus does not extend to nanoscale connectivity between neurons. Alternative approaches have used matrix views [HVU*22] or visual abstractions in 2D that retain some morphological features, such as neuron branches [AABS*14] or arborizations [SBS*13]. All the above approaches are compact data representations that allow scientists to get a quick overview of the data. However, the three-dimensional morphology of neurons and their spatial relations in the data is lost. Furthermore, most prior works focus on either very small volumes and, thereby, truncated brain networks [BAAK*13, AABS*14] or use lower resolution datasets and heuristics to generate synapse positions [SBS*13]. Most recently, Plaza et al. [PCD*22] developed neuPrint, a tool to query neuronal connectivity quickly in the browser. neuPrint uses the Cypher language [FGG*18] to query for MIs but does not focus on visually highlighting the connectivity in the MIs. Our tool builds on the neuPrint data infrastructure but provides an intuitive sketching interface for specifying motifs visually.

Visual Graph Queries. Visual query interfaces have been used in many application areas and allow users to specify queries in an intuitive way [Ege97, CWW*10, MA18]. Cuenca et al. [CSIP22] propose Vertigo, an approach to construct and suggest graph queries and explore their results in multi-layer networks. Vertigo visualizes all detected subgraphs as an overlayed heatmap to a graph drawing. This approach, however, is not feasible for large connectomic graphs, and all information on the 3D morphology of neurons would be lost. Vigor [PHE*18] focuses on effectively summarizing

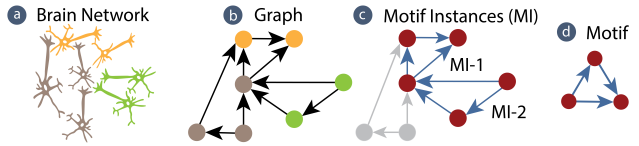

Figure 3: ***Motifs in brain networks.*** *(a) Brain networks consist of neurons that connect through synapses. (b) Graph representation of the same brain network. (c) Network motifs are recurrent subgraphs in the larger brain network. For example, this connectivity graph contains two instances of a feedforward motif (d).*

subgraph queries by grouping results by node features and structural result similarity. In *Vimo*, users can use structural similarity constraints directly in the query interface.

Visualization of Network Motifs. Visualization of motifs has been proposed in different domains. For instance, MAVisto [SS05] supports visual motif analysis in biological networks. However, MIs are only visualized as node-link diagrams, and 3D shapes of biological objects are hidden. Dunne et al. [DS13] simplify node-link diagrams of motifs using glyphs. Those simplification techniques do not extend to complex spatial data like neuronal structures. For dynamic networks, Cakmak et al. [CFJ*22] compare motif significance profiles over time by plotting them as a time series.

Visual Abstraction of Tree-Like Structures. Different visual abstractions have been proposed to analyze neurons and tree-like structures. Hu et al. [HBMK22] project a complex 3D tree-like structure onto a 2D plane while avoiding overlaps. However, their method does not scale to groups of treelike structures, as needed for neuronal motif analysis. Mohammed et al. [MAAB*18] use a continuous 2D abstraction space to analyze astrocytes and neurons. Neurolines [AABS*14] uses a 2D subway map metaphor to display neurons. However, both approaches focus on relatively small subvolumes and would not visually scale to large connectivity graphs.

3. Biological Background

Brain anatomy. Brain tissue primarily consists of long tubular tree-like nerve cells or *neurons*, which transmit signals to each other via *synapses*. A single neuron can connect to hundreds or thousands of other neurons. The high interconnectivity of neurons combined with their complex morphology results in tangled three-dimensional brain networks that are difficult to analyze. Different neuron types have unique morphological properties and connectivity preferences. Most brains of model organisms, such as the fruit fly (*Drosophila Melanogaster*), are further divided into spatial regions with distinct anatomical and functional properties.

Brain networks and motifs. Connectomic datasets with tens or hundreds of thousands of neurons can be interpreted as large *directed graphs*, with neurons as nodes and synapses forming edges between the nodes. Recent advances in microscopy and computational neuroscience have revealed characteristic non-random patterns in neural networks of invertebrates and mammalian brains, especially in the cerebral cortex. The recurring wiring patterns [MSOI*02], termed *network motifs*, suggest the hypothesis that neuronal connections are arranged in some basic building blocks with hierarchical order [SK04]. Fig. 3 shows how an example brain network and its graph representation.

Connectomics Data. Analyzing neuronal tissue at the level of synapses requires ultra-high-resolution imaging techniques such as serial-section scanning electron microscopy (SEM), which can produce more than 1 petabyte of data for each imaged cubic millimeter [SCJB*21] of tissue. In this paper, we demonstrate our visual motif query and analysis approach on the FlyEM hemibrain, one of the largest open-source connectomic datasets. It contains the entire central region of the fruit fly brain, including 25,000 proofread neurons classified into more than 5,000 types, over 20 million synapses, 13 main brain regions, and over 200 sub-brain regions [SXJ*20]. It is a major resource in the Drosophila neuroscience community, which has led to exciting scientific discoveries already [LLM*20,HHF*21,SBS*21b]. *Vimo* processes the connectivity graph, 3D neuronal skeletons, and spatial synapse locations of the hemibrain dataset (see Sec. 9).

4. Goal & Task Analysis

The idea of *Vimo* originated from meetings with our neuroscience collaborators, who were excited about newly available EM datasets, but needed the means to analyze the synapse-level connectivity within this data on a larger scale. The scientists want to identify and explore motifs and synaptic chains without being overwhelmed by the complex 3D structure of intertwined neurons. At the same time, they need to see motifs in the original 3D volume space to understand the spatial relation of neurons and their synapses.

Following the problem-driven design study approach [SMM12], we identified a set of domain goals and tasks in semi-structured interviews with five experienced neuroscientists from the Harvard Center for Brain Science, HHMI Janelia, and the Zuckerman Institute at Columbia University. All five scientists have multiple years of experience with analyzing neuronal circuits reconstructed from EM image data. Three scientists are experts in analyzing connectivity patterns in the brain of Drosophila. Additionally, we presented an early prototype of the tool to dozens of researchers at an international connectomics conference to refine our goals and tasks.

4.1. Domain Goals

The neuroscientists' main objective is finding biologically relevant motifs in large neuronal networks. Further, they want to analyze interesting motif instances visually in more detail to fully understand how the motif's connectivity relates to each neuron's morphology.

G1 - Motif identification in large brain connectivity data. Our collaborators want to search large brain networks for instances of a wide range of connectivity motifs based on **neuronal connectivity** and additional **biological constraints** on the involved neurons and synapses. Neuroscientists want to specify details such as the *brain region* a neuron trajects, the *neuron type*, or set the *number of synapses* a neuron makes in a specific brain region. For example, a specific type of ring neuron in the fly brain is involved in action selection tasks [BKHH21], and scientists want to analyze motifs involving this specific neuron type. Biological constraints ensure the expressiveness of motif queries when studying such behaviors.

G2 - Analysis of motif instances. Since a motif search in a large brain network might result in dozens to thousands of hits, our collaborators need to be able to easily **identify interesting motif instances (i.e., *MIs*)** and then explore them in high detail. Neurons

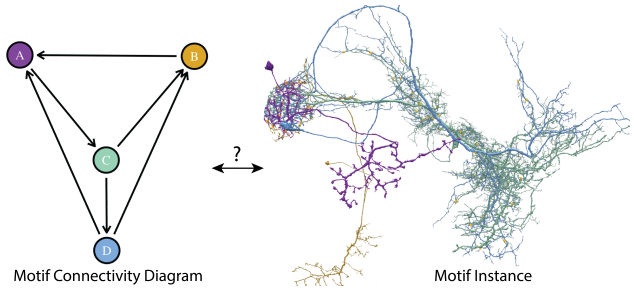

Figure 4: ***Understanding connectivity.*** *Understanding motif connectivity in the complex 3D morphology of neurons in an MI is not trivial. Domain experts need better tools to understand how a connectivity motif is expressed in a set of neurons.*

form complex three-dimensional shapes, span long distances, and connect with up to thousands of other neurons. Therefore, our collaborators previously struggled to **identify how a set of neurons forms a motif** and how the abstract connectivity motif maps to the actual anatomical structure of the involved neurons and synapses (see Fig. 4). However, this understanding is crucial for following information flow along the neurons. In particular, our collaborators have questions such as *"Which branches and subbranches of the neuron make up the motif?"* or *"Are several spatially distinct parts of a neuron involved in the same motif?"*.

G3 - Identification and analysis of motif chains. In addition to exploring single MIs, our collaborators are interested in exploring motif chains. Starting from a motif of interest and a seed neuron, they want to **explore if the neuron is involved in different instances of the same motif**. This allows scientists to speculate about the importance of certain neurons in a motif. Furthermore, our collaborators are interested in motif-based synaptic chains. Starting from a seed MI, they want to **follow chains of computation** to see if the same motif is used consecutively over a larger number of neurons. This analysis can give more holistic insights into information flow (e.g., from visual input neurons to the central brain of Drosophila).

Scalability Considerations. To reach the above goals, the main requirement of our application is *scalability*. The datasets our collaborators want to analyze contain tens of thousands of neurons and millions of synapses. Therefore, the entire analysis pipeline, including data access, algorithms, and interaction metaphors, need to support large data and scale to future datasets.

4.2. Tasks

Based on the above domain goals, we have derived a set of analysis tasks *Vimo* needs to support:

T1 - Query for connectivity motifs. Scientists need an *intuitive* way to specify motifs and biological constraints for queries (**G1**).

T2 - Identify interesting motif instances. Scientists need to be able to explore query results to identify interesting MIs (**G2**).

T3 - Explore the spatial and anatomical context of MIs. For an interesting MI, scientists want to explore the anatomy of its neurons and synapses in the context of the surrounding brain region (**G2**).

T4 - Identify mapping between neuron morphology and motif connectivity. After their initial exploration of an MI, scientists need to identify the parts of the neurons that make up the motif and analyze the detailed connectivity of the motif instance (**G2**).

T5 - Query and identify connected multi-motif instances. Starting from the neurons of a selected motif instance, scientists want to explore whether the same neurons are involved in other instances of the same underlying motif (**G3**).

T6 - Trace synaptic chains starting from seed MI. Starting from a seed MI, scientists want to identify and follow synaptic chains made up of repeating specific motifs (**G3**).

5. *Vimo* Design and Workflow

Our main goal for *Vimo* is to provide neuroscientists with the means to easily explore the connectivity of their large datasets and allow them to understand the underlying network structure of neurons with their complex anatomical structure. To achieve this, we designed a workflow that offers intuitive user interactions and visualizations that highlight the connectivity of the data while keeping the underlying anatomical data undistorted (see Fig. 5).

Specifically, we designed an *interactive visual query interface based on sketching* to search for network motifs in the data. The interface is aimed at domain experts with no programming background and allows users to specify motif connectivity and biological constraints (**G1**). The main difficulty of connectivity analysis in high-resolution connectomic data is the high complexity of both neuron morphology and connectivity. Neurons are densely arranged in brain tissue and often highly intertwined. Synapses between two neurons might be clustered in one region or spread out along the length of the neurons. Therefore, *Vimo* offers a *3D view* to display neurons and synapses of a selected MI in detail. This allows scientists to understand overall neuron shape and how neurons of a motif are intertwined. To support neuroscientists in the understanding of both morphology and connectivity, we designed a method for *gradually highlighting neuronal connectivity*, similar to continuous visual abstractions. Users can focus more and more on the underlying connectivity of the data while still retaining access to the morphology of neurons (**G2**). *Vimo* allows users to identify and follow interesting synaptic chains created by the repeated appearance of a motif in the data. To reduce the search space for synaptic chains, we let users start with a seed motif or neuron and support a user-driven exploration of the data (**G3**). Finally, throughout the design of *Vimo* we focused on scalability by using datasets hosted in the cloud and only downloading small subsets to the user's machine during runtime (see Sec. 9).

6. Interactive Motif Queries

In contrast to previous efforts in motif analysis that rely on programming languages [MRJ*21, PCD*22], *Vimo* provides a visual sketching interface to create queries. This gives scientists an intuitive approach to search for motifs. Additionally, *Vimo* provides real-time feedback on the significance of a sketched motif in the brain network to guide the user during the analysis process.

6.1. Motif Sketching

In *Vimo*, users search for motif instances by sketching an exemplar. They draw a set of nodes and edges to define the motif (i.e., neurons

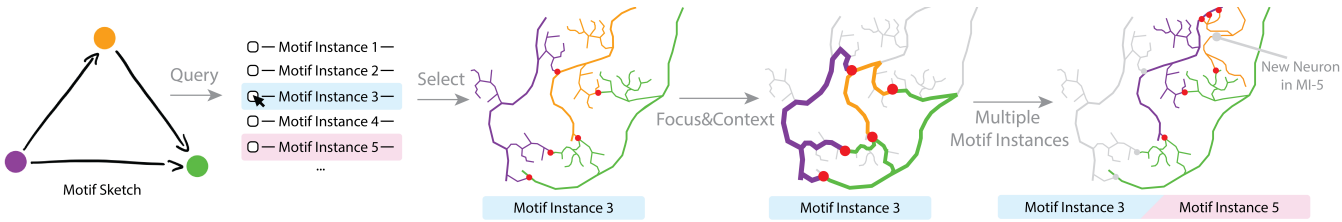

Figure 5: Vimo *workflow.* *Users query for motifs based on a sketch and select motif instances for further investigation. While exploring an MI, users can adjust the visualization to gradually highlight the connectivity of neurons, or continue to explore multiple MIs.*

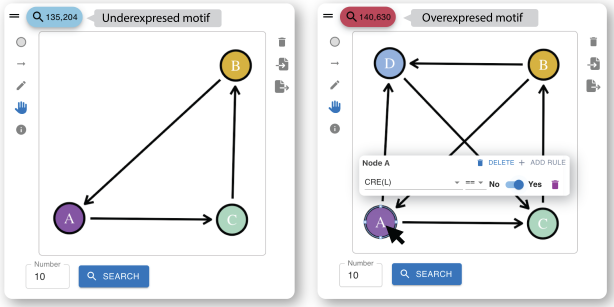

Figure 6: *Motif sketching interface.* *Users draw nodes and edges of a motif (left) and define biological constraints on the nodes and edges (right). Additionally,* Vimo *gives real-time feedback on the absolute count of a sketched motif in the dataset and by indicating whether a motif is over- or under-represented (red and blue labels).*

and the connections between them) (see Fig. 6), specify additional biological constraints, and inspect the results of their motif query.

Defining Constraints. To create biologically meaningful queries, users can interactively set constraints on the nodes and edges of the motif sketch. Our query interface supports constraints on the *neuron's type*, the *spatial location and brain region* of neurons and synapses, the *strength* of a connection, and the *neuron's ID* (**T1**). For instance, users can define one of over 5,000 neuron types or trajectories through over 200 brain regions in the hemibrain dataset. We use an intuitive and expressive query builder interface to define one or multiple constraints per node and edge (see Fig. 6). Autocompletion of types and brain regions further helps users quickly select from thousands of available options.

Guided Sketching. Deciding which motifs to study in greater detail is not always obvious to neuroscientists. During formative interviews, experts expressed interest in analyzing particularly common or rare motifs in the network. Therefore, as the user is sketching a new motif, we provide real-time feedback about the significance of the sketched motif. In particular, *Vimo* shows the number of occurrences of the motif in the brain network and whether the motif is estimated to be over- or under-expressed in the network (**T2**).

Counting the number of occurrences of a motif in a large graph in real-time is computationally infeasible. Therefore, we precompute motif counts with a subgraph enumeration technique by Matejek et al. [MWC*22] on a high-performance compute cluster. At run-time, we perform a simple look-up to display the number of occurrences of a sketched motif in the brain network (see Fig. 6).

We also indicate the over- or under-expression of a motif in the brain network using a heuristic approach. We estimate a motif as over-expressed if its occurrence in the brain network is higher than in a random network. It is under-expressed if it occurs less frequently in the brain than in the random network. To approximate the motif count in a random network, we use Erdös models [IMK*03]. We indicate over- or under-expression with a red or blue badge in the sketching panel, respectively (see Fig. 6).

Running Motif Queries. *Vimo* automatically translates the visual sketch into a *DotMotif* [MRJ*21] string in real-time. Once users are satisfied with their sketch, we send the query string to the neuPrint [PCD*22] graph database to get a list of motif instances. Note that any graph database supporting *DotMotif* queries can be used. See Sec. 6.2 for the scalability of motif queries.

Inspecting Motif Query Results. *Vimo* displays the results as a set of MIs in a list view (see Fig. 10b). To guide users in selecting a motif instance for further analysis, we show summarizing features for each neuron in a motif instance. In particular, we display the neuron's type, Id, and proofreading status (**T2**). Clicking on an MI shows the 3D models of the neurons and their synapses in the main view of the *Vimo* interface.

Reproducing and Sharing Sketches. Users can import and export motif sketches as JSON files. This helps researchers to reproduce their motif queries at later times and facilitates sharing interesting discoveries with colleagues. A set of interesting motif sketches from the case study is available in the supplementary material.

6.2. Computational Challenges

Subgraph isomorphism searches are computationally expensive [MWC*22, RPS*22]. For example, verifying the existence of a motif in a larger network is an NP-complete problem [Coo71]. We use two strategies to limit the computational complexity of queries. First, users can set specific *biological constraints* on the nodes and edges, reducing the search space significantly. For example, neuroscientists are interested in motifs involving external ring neuron *(ExR1)* types. The hemibrain dataset [SXJ*20] contains four neurons of type *ExR1*, drastically reducing the search space by only querying the neighbors of those four *ExR1* neurons. Second, users can *limit the number of MIs* returned by a query. Keeping this number low allows the search algorithm to terminate early without finding all MIs in the network. Visually inspecting a small number of MIs is often satisfactory for exploratory or initial analysis. We use heuristics, like the number of nodes in the motif and the number of edges without specific constraints, to estimate if a motif query will run longer than 20 seconds, in which case we warn the user.

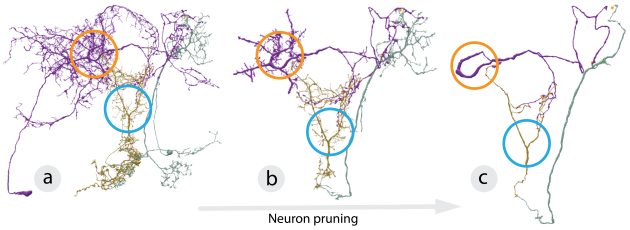

Figure 7: ***Motif pruning.*** Vimo *gradually emphasizes neuron connectivity in MIs. We first show the full 3D morphology (a) and subsequently prune all branches unrelated to the motif (b) until only essential branches remain (c). In the process, key features of the motif become visually more prominent (colored circles).*

7. Motif Analysis

Most connectome analyses require a detailed understanding of neuron wiring. However, due to the complex morphology of neurons, it is hard to map the motif connectivity to a 3D visualization of the neurons (see Fig. 4). Previous visualization tools either use plain node-link diagrams [SCHT09, BAAK*13] or create planar embeddings of neurons [AABS*14]. However, neither node-link diagrams nor planar visual encodings can accurately show spatial relationships between neurons. 2D projections of neurons abstract their spatial entanglement, which is relevant to understand connectivity. Based on those observations, we designed a spatial exploration and focus&context method that gradually emphasizes motif connectivity in the 3D representation of a motif instance to facilitate a better understanding of the data. Additionally, we have designed a set of targeted interactions that further highlight connectivity without distorting neuron morphology.

7.1. Spatial Exploration

As the first step in the exploration process, *Vimo* supports the interactive inspection of the 3D spatial morphology of all neurons of a motif instance (**T3**). To ensure scalability, we render neurons based on their skeleton. Skeleton representations of neurons are faster to download during runtime than meshes, as they require less data while retaining the essential morphological details of neurons. For each skeleton node, *Vimo* uses the neuron's diameter to adjust the skeleton thickness, leading to more accurate visual representations. To allow users to quickly identify the role of a neuron in the motif, we further color each neuron corresponding to its color in the motif sketch. For context and to enhance a user's spatial orientation, the 3D view in *Vimo* can further show the outlines of brain regions.

7.2. Gradual Motif Highlighting and Abstraction

Our visual abstraction approach contains three main steps, all aimed at gradually highlighting motif connectivity, to help users understand how neurons form a certain motif (**T4**). Users can gradually move between the steps by dragging a slider in the user interface (see Fig. 10e), which results in a continuous animation from one highlighting- and abstraction level to the next.

Neuron pruning. In the first step, we aim to highlight motif connectivity by visually removing those parts of a neuron that are not involved in the connectivity motif. Users can gradually peel away all parts of the neuron that are not in the motif, which reduces visual clutter and focuses the user on the important parts of a neuron in relation to the motif. We start with a 3D skeleton representation of all neurons in the motif instance (see Fig. 7a). First, we identify all non-motif branches (i.e., neuron branches with no synapses involved in the motif). Next, we compute the geodesic distance of every skeleton vertex in a non-motif branch to its closest motif synapse. This allows us to gradually prune non-motif branches based on their distance to the motif by moving a slider until only branches with motif synapses remain (see Fig. 7b,c). After pruning all non-motif branches, the user can see the essence of all neurons that make up the motif (see Fig. 7c).

Exploded view. In the second step, we further reduce the visual complexity resulting from entangled neurons. We spatially pull all neurons apart into an exploded view after all non-motif branches are pruned (see Fig. 8b). This allows scientists to study the morphology and branching patterns of individual neurons and better see where synapses involved in a motif are located. To evenly distribute the neurons in space, we use the Saff and Kuijlaars algorithm [SK97] to compute the directions of the explosions. The algorithm evenly distributes n points on a unit sphere. We then compute the explosion directions by taking vectors from the sphere's center to each sampled point. In the exploded view, all pre- and post-synaptic sites between motif neurons are marked with spheres colored similar to their synaptic partner neuron (see Fig. 8b). This helps the user quickly grasp the neurons' connectivity, even as the neurons are spatially apart.

Connectivity Visualization. In the third step, we highlight connectivity in the exploded view. We initially draw lines between the pre- and post-synaptic sites of the neurons (see Fig. 8 c,d). We use two strategies to highlight important connectivity features and avoid visual clutter. First, we use hierarchical clustering of synapses and 3D bundling to aggregate lines. We choose a hierarchically bundling strategy as it can visualize different levels of synapse clusters and how they are distributed along the neurons. Based on feedback from our collaborators, we cluster synapses based on their spatial proximity on the neuron and based on which neuron they connect to. The user can set the bundling strength via the focus&context slider. Alternatively to the clustering approach, the user can also decide to only see the connecting lines for user-selected synapses.

Design Alternatives. In an initial version of *Vimo*, we gradually moved from the pruned neuron view all the way to a node-link view by abstracting neurons slowly into nodes and collapsing synapses to form the lines between the nodes. We hoped this would help scientists better see the relation between the motif and the involved neurons. However, we ultimately abandoned this idea since scientists could already see the abstract motif in the sketching interface and had no use of such a simplified view of the motif in their analysis. They are interested in seeing more nuanced anatomical details.

7.3. Interaction

We offer several further interactions for visual motif analysis.

Synapse highlighting. In *Vimo*, synapses are drawn as small spheres between the pre- and post-synaptic sites of the connected neurons. Visually highlighting all synapses between two neurons

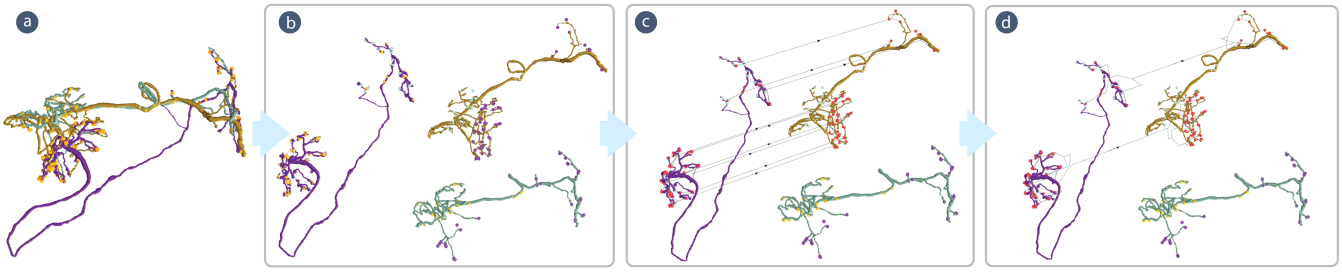

Figure 8: ***Exploded view and connectivity visualization.*** *After neurons are fully pruned (a), we explode the view (b) to reduce visual complexity due to neuron entanglement.* Vimo *gradually clusters synapses and bundles directed edges between pre- and post-synaptic sites to further reduce visual clutter (c, d). Two bundles indicate two distinct clusters of synapses between the purple and the yellow neuron (d).*

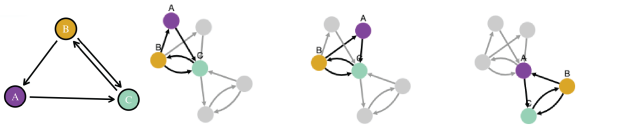

Figure 9: ***Visualizing multi-motifs.*** Vimo *supports exploring connections between multiple motif instances. For instance, this set of six neurons forms the sketched motif three times.*

provides a simple yet effective visual clue to study where neurons connect. A set of synapses is either highlighted by clicking on a synapse sphere in the 3D view or by selecting an edge in the sketching panel or the summary view (see Fig. 10d).

Showing brain regions. Interactively rendering the surface of small brain regions provides orientation within the brain and allows users to verify if a selected motif instance adheres to the constraints specified in the sketch. We show semi-transparent surfaces of the brain regions overlayed to the 3D renderings of the neurons. Users can interactivley select which brain regions to show and quickly enable and disable the renderings.

Graying out non-motif branches. While neuron pruning (see Sec. 7.2) helps remove unrelated branches to a motif instance, it removes context about the motif neurons that might be necessary during analysis. Therefore, *Vimo* allows graying out all non-motif branches of a motif instance to highlight important parts of the neurons and show the context of all other neuron branches.

Further Analysis. Once scientists observe interesting motif characteristics in an exploratory analysis session in *Vimo*, they need to perform an in-depth analysis on a set of MIs. This involves in-depth statistical analyses and studying synapse distributions. *Vimo* supports this step by integrating tightly with neuPrint [PCD*22], which provides a set of analysis tools for neurons. Users can access neuPrint data for all neurons of an MI by using the context menu.

8. Motif-based Synaptic Chains

Analyzing a single MI at a time provides only a partial view of the connectivity of involved neurons. To study how a motif instance is embedded in the larger network, scientists need to visualize multiple connected MIs simultaneously (see Fig. 9).

8.1. Multi-Motif Analysis

Vimo supports the analysis of synaptic chains in two ways:

Neuron-centric analysis. First, domain experts are interested if a neuron forms the same motif with multiple partners (**T5**). For instance, in Fig. 9, neuron B forms the same motif with two neurons of type A (MI-1, MI-2). Users can specify a *seed neuron* in the motif sketching interface to ensure that only motif instances involving that particular neuron are returned.

Synaptic pathway analysis. Second, neuroscientists are interested in following synaptic pathways that repeatedly include a sketched motif. For example, MI-2 and MI-3 form such a synaptic pathway in Fig. 9. To query for those, users start from a seed motif and set the ID of a sink neuron in the motif instance to a source neuron in the motif sketch through a context menu (**T6**). This strategy aids in continuously building up motif-based synaptic pathways downstream or upstream from a seed motif instance.

8.2. Multi-Motif Visualization

To support analyzing multiple motif instances, *Vimo* uses three strategies to visually guide the analysis.

Connectivity Summary. *Vimo* provides an abstract overview of the connectivity of all selected MIs in a small node-link diagram (see Fig. 10d). The nodes and edges of the focused motif instance are colored, while all other elements are grayed out. This overview can help the user to stay oriented in the 3D view. Selecting an edge in the summary view also highlights all corresponding synapses in the 3D view and the corresponding edge in the sketch panel.

Highlighting motif instances. Inspecting one motif instance in 3D can already be overwhelming due to the complex morphology of neurons. *Vimo* always focuses on a particular MI, while all other unrelated neurons are grayed out to highlight the neurons of the MI in focus. Users can quickly switch focus by clicking on a different MI in the list view.

Multi motif abstraction. We designed the continuous focus&context approach (see Sec. 7.2) to scale to multiple motif instances. Pruning and exploding multiple motif instances simultaneously helps domain experts to better understand how those instances wire to form a synaptic chain.

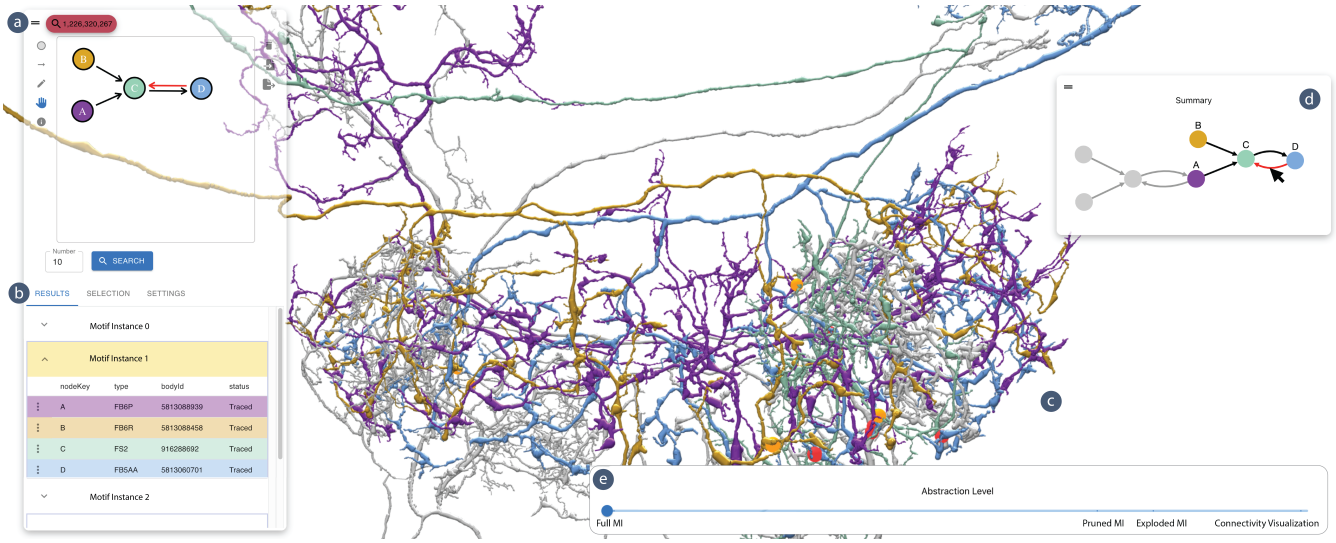

Figure 10: ***Overview of the* Vimo *user interface.*** *Motif sketch of a participant in the user study (a) and list view of resulting MIs (b). The central view shows 3D renderings of all selected MIs (c). The multi-motif summary view visualizes the relationship between the selected motif instances when following motif chains (d). Users control the focus&context abstraction level using the interactive slider (e).*

9. Data and Implementation

Data. *Vimo* requires a connectivity network and proofread reconstructions of neurons in the form of 3D skeletons and synapses, including their spatial locations and pre- and post-synaptic partners. Meta-data, like neuron types and brain region trajectory information, are used to increase the expressivity of motif queries but are not required. Precomputed motif counts can guide motif sketching (see Sec. 6.1). *Vimo* expects data in the neuPrint format [PCD*22]. neuPrint currently hosts four datasets (3 hemibrain versions and the fib19:v1.0 dataset), and new datasets are expected on the same platform in the future. For the development and evaluation of this project, we used the *hemibrain v1.2.1* dataset.

Scalability. Interactivley exploring tera- and petascale connectome datasets requires scalable tool architectures. We leverage different data representations with strong compression rates to achieve scalability. For instance, the electron microscopy imaging data of the hemibrain dataset is 26 TB in size [SXJ*20]. In contrast, the skeleton representations of the reconstructed neurons require only ∼10 GB, while the pure connectivity graph compresses the data to ∼25 MB [SXJ*20]. As a result, the data size required for certain analyses is reduced by a factor of a million by transforming the imaging data into a connectivity network. We exploit these compression rates by purposefully choosing the data representation for each step of the analysis workflow. For instance, *Vimo* queries the compact connectivity network for motifs. Based on the detected and selected motif instances, *Vimo* downloads a small set of the neuronal skeletons and synapses from the neuPrint server [PCD*22] or from a pre-computed cache during runtime. Those strategies enable domain experts to use *Vimo* on consumer-level hardware without specific requirements for RAM and graphics hardware.

Implementation. *Vimo* is implemented as a web application, using a React-based frontend and a Python-based backend. *Vimo* builds on existing software frameworks like *NaVis* [SBS*21a] for data processing, and a modified version of *SharkViewer* [WBH14] for interactive 3D rendering. The hemibrain dataset [SXJ*20] is hosted remotely in the *neuPrint* ecosytem [PCD*22]. Motif sketching is implemented using the *Paper.js* library. *Vimo* translates the visual sketch to an optimized *Cypher* query [FGG*18] using the *Dotmotif* [MRJ*21] language as an intermediate step. This *Cypher* query is then sent to a remote graph database to determine a list of motif instances. *Vimo* is open-source, and we offer detailed instructions for users in our tutorials.

10. Evaluation

We report on an in-depth case study and qualitative user study to evaluate the usability and usefulness of *Vimo*.

Participants. We evaluated *Vimo* with 7 domain experts (P1 - P7, 3 male, 4 female) from the Harvard Center for Brain Sciences and HHMI Janelia research campus. Two participants are also co-authors. To limit participants' time commitment, three participants performed the case study, and the remaining four participants completed the user study. All participants are experts in analyzing neuronal circuits reconstructed from EM image data, four are experts in Drosophila connectomics (6 postdoctoral researchers and 1 Ph.D. student in neuroscience). None of the participants use interactive tools for motif analysis, even though 6 out of 7 participants rate motif analysis as important for their research.

Setup. We met with each participant for 90 minutes in person or on Zoom. After a short introduction to the tool, all users, who had no hands-on experience with *Vimo*, steered the tool themselves. We asked all participants to think out loud to capture their thoughts.

10.1. Case Study: Exploratory Analysis

We report on an exploratory case study with domain experts specializing in analyzing neuronal circuits in the Drosophila brain. We provided no specific tasks, as we wanted to test what types of analyses an experienced neuroscientist would conduct. We describe two motifs in detail that were analyzed by one expert during the session.

Feedforward outputs of ExR neurons. First, the expert searched for a familiar connectivity motif to verify the tool's reliability. They started by sketching a feed-forward motif involving an external ring (*ExR*) neuron, an ellipsoidal body (*EB*) neuron, and a neuron without any constraints **(T1)**. The motif can be reproduced by loading this sketch. The expert iteratively refined their sketch by adding more constraints to the nodes and edges until they found an MI that matched the desired characteristics. Next, they selected an MI that included one *ExR* neuron and two *EPG* neurons (Ellipsoid body - Protocerebral bridge - Gall) **(T2)**. After inspecting the 3D renderings, they used the summary view to highlight the synapses between the *ExR* neuron and both *EPG* neurons (see Fig. 10d). The expert indicated that this helped confirm that the *ExR* neuron forms synapses with each *EPG* neuron on different arbors **(T3)**. They decided to study this observation in more detail using the focus&context slider and first pruned all unrelated motif branches of the neurons. Next, they iteratively moved back and forth from the non-exploded view to the exploded view to understand better how the neurons entangle at areas of strong synaptic connectivity. Next, they studied the bundled lines between the pre-synaptic sites of the *ExR* neuron and one of the *EPG* neurons. They stated that the exploded view helped them to find a previously unknown connection and was especially helpful for quickly identifying which synapses might be considered biological noise as they are distant to strong synapse clusters **(T4)**. Finally, the expert was interested in other instances of the sketched feed-forward network that also involve the previously studied *ExR* neuron and one of the *EPG* neurons. Hence, they searched the query results for a motif instance that included these particular neurons and added this MI to the 3D view to analyze how these multiple motif instances connect **(T5)**. They first focused on each MI individually to better understand the spatial relationships between the two MIs. Finally, they used neuron pruning to compare the connectivity of both motif instances.

Circular connections in visual input neurons. Next, the neuroscientist sketched a motif forming a circular connection between three visual input neurons, specifically tuberculo-bulbar (*TuBu*) neurons and ellipsoid body ring (*ER*) neurons **(T1)**. The motif sketch and the studied motif instance are available for reproducible results. Based on the motif counts in the sketch panel, the expert found that circular connections are underrepresented in the dataset, making it interesting for detailed analysis **(T2)**. After analyzing an MI in 3D, the expert identified that all synapses are clustered tightly at a specific spatial location **(T3, T4)**. Based on this observation, the scientist was interested if other neurons of the same type formed the same motif but expressed stronger connection strengths. Hence, they increased the synapse strength constraints in the sketch and found another MI with an even stronger synapse cluster close to the previously observed cluster **(T1, T2)**. As a final step, the expert used the exploded view to study the internal structure of the cluster and inspected the bundled lines to learn how the circular motif is expressed within the cluster **(T4)**.

10.2. Qualitative User Study

To evaluate the usability and usefulness of *Vimo* in a qualitative user study, we asked participants to perform two tasks: motif sketching and analyzing motif connectivity with our focus&context approach.

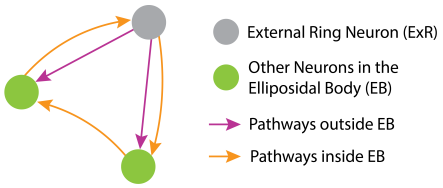

Figure 11: ***Motif sketching task.*** *Study participants had to sketch this known biological motif between* ExR *neurons and other neurons in the ellipsoidal body (*EB*) of Drosophila [HHF*21].*

10.3. Task 1: Motif Sketching and Querying

In the first task, we asked participants to sketch and query for a connectivity motif recently discovered by Hulse et al. [HHF*21] **(T1)**. The motif describes *ExR* neurons and other *EB* neurons that form connections inside and outside the *EB* and contributes to the context-dependent action selection behavior of Drosophila [HHF*21]. A correct motif sketch and a related motif instance are available to reproduce results. We provided a schematic illustration of the motif and its constraints (see Fig. 11) to all participants. Sketching the connectivity between the motif neurons was straightforward for all participants, while defining node and edge constraints required a learning process. Two participants needed help from the study conductor to specify appropriate constraints. We observed that the performance of defining constraints depends on the participant's familiarity with fly brain anatomy and knowledge about Drosophila brain regions. Participants familiar with the dataset could easily identify how to translate the instruction *'outside EB pathway'* into an edge constraint. Our survey results show that all four study participants find the motif sketching interface useful (see Fig. 12, Q2). However, for querying large motifs involving more neurons (e.g., $n \geq 6$), only 3 out of 4 participants find motif sketching useful because of visual clutter in the sketching interface. During the pilot study, we found that neuron-type wildcards can improve the utility for defining node constraints. Therefore, we added this functionality which was then widely used for the remainder of the user study. Additionally, participants suggested changing the default setting for synapse strength constraints to avoid repetitive interactions.

10.4. Task 2: Analyzing Motif Connectivity in 3D Data

In the second task, we provided users with a motif and the 3D view of a motif instance and asked them to identify the main motif connectivity in the three-dimensional data **(T3, T4)**. We tested two conditions: In the first condition, participants had access to the full functionality of *Vimo*, including our focus&context technique. In the second condition, participants could not use our focus&context method. We used two motif instances which we counterbalanced between conditions. Every participant had to perform the task with both conditions. To test their understanding of the motif connectivity in 3D, we asked participants to draw an illustration of the neuron branches involved in the motif and their connections for both conditions. We observed that the use of our focus&context technique led to less cluttered illustrations by the participants, indicating a clearer and better understanding of motif connectivity due to interactive simplification. We show example user illustrations in the supplemental material. Additionally, we collected sur-

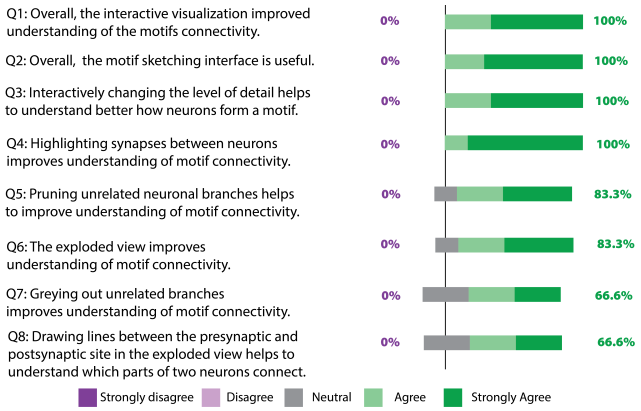

Figure 12: Vimo *user ratings.* *We show neutral responses in gray, positive responses on the right in green and the percentage of participants who responded positively.*

vey responses from all participants about our interactive motif abstraction approach (see Fig. 12), demonstrating the usefulness of the different abstraction components.

10.5. Findings

***Vimo* leads to fast, iterative analysis.** We observed that users often query a specific motif but forget to specify certain constraints in the motif sketch. *Vimo* supports users to quickly iterate on their motif sketches and queries, leading to more relevant motif analyses.

Biological constraints are essential for motif analysis. We found biological constraints to be essential for expressive motif queries. Specifically, wildcards for neuron types were perceived well, constraining a neuron to a group of types. For instance, the *ExR** type requires a neuron to be in any of the *ExR1 - ExR8* types.

Exploded views improve spatial motif understanding. The gradual transition of neurons into the exploded view helped users understand how neurons entangle. Understanding the complex entanglement is crucial for analysis but also an obstacle for mentally mapping the motif graph structure to the 3D visualizations of neurons.

***Vimo* improves the state of the art.** All study participants agreed that *Vimo* improves their motif analysis workflow. Users particularly liked the interactive motif sketching together with neuron pruning and synapse highlighting for their visual analysis in 3D.

11. Discussion

User expertise for high-resolution connectivity analysis. It was challenging to find qualified experts for the user study. Many neuroscience labs are interested in connectivity analysis at the nanometer scale, however, few have done it yet. Datasets have only recently become available, and with a lack of scalable computational tools for high-resolution connectivity analysis, many scientists have not yet started on this endeavor. We hope that *Vimo* can reduce the barrier of entry for this type of research and attract more labs and researchers to analyze high-resolution connectivity.

Limitations. *Vimo* focuses on the interactive analysis of motifs and quick, iterative refinement of motif queries. Therefore, the strength of our tool is in analyzing relatively small motifs, with a limited number of nodes. While our visualization approach scales to larger motifs, running large motif queries becomes computationally expensive, and would hinder the interactivity of our tool. Precomputing motif queries and caching the results would circumvent this issue. Further, while *Vimo* supports the visual analysis of multiple connected motif instances, large-scale comparisons of motif instances distributed across the entire dataset are not yet supported. Finally, in *Vimo*'s current design, researchers need a prior hypothesis about relevant network motifs before sketching a pattern. In other types of analyses, researchers start from a fixed set of neurons and are interested in the set of expressed connectivity motifs. However, even a small number of neurons are involved in an immensely large set of different motifs. This exploratory approach is inverse to *Vimo* and not yet supported in our tool.

Tradeoff between accuracy and visual abstractions. A main design decision of our approach is that we do not distort neuron anatomy in any way, but rather want to enhance the user's perception of the motif connectivity in the data. Many previous approaches have focused on visual abstractions that simplify neurons and neuron anatomy or use 2D projections of the 3D connectivity graph [AABS*14, MAAB*18]. We take a complementary approach and focus on highlighting connectivity in the original data. This comes at the cost of a higher cognitive load than using simplified 2D representations, however, it allows scientists to better understand the detailed spatial make-up of a motif and its neurons.

12. Conclusion and Future Work

In the future, as more large-scale and proofread datasets become available [SXJ*20, MABB*21, SCJB*21, TMB*22, DMM*22], we want to extend *Vimo* to support data from other organisms, such as mice and humans. Generally, all our approaches generalize, but small differences apply. For instance, in the human data, fewer neurons form multisynaptic connections compared to the brain of Drosophila, making motif connectivity strength a less powerful query constraint. However, in contrast to the fly brain, neurites in the human brain can be labeled more clearly as axonic nor dendritic, which would allow us to emphasize the direction of information flow in our focus&context method. Furthermore, with these new datasets, comparative visual motif analysis will soon be within reach. Researchers can already identify a one-to-one correspondence between neurons across the different specimens in the brain of Drosophila. Thus, future tools should support analyses into whether the same neurons across specimens form similar motifs.

With *Vimo*, we have taken a first step towards scalable visual motif analysis for nanoscale brain data. The core idea of our approach is to enable scientists to quickly and intuitively sketch motifs they are interested in and to allow them to add biological constraints to their queries. We support a detailed motif analysis in the original 3D space, using a focus&context approach. We give users a better understanding of their data by allowing them to gradually highlight motif connectivity in relation to the three-dimensional structure and arrangement of neurons and synapses in the brain. We believe that *Vimo* is a first step towards neuronal pathway analysis at a larger scale, where structural and functional data are combined for a better understanding of the brain.

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