GANGLIA: A tool for designing customized neuron circuit patterns

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Abstract. Current biological neural controllers in biohybrid robotics rely on networks of self-assembled neurons. However, to be able to reproducibly create neuron circuits optimized for specific functions, the connections that the neurons form need to be controlled. Towards addressing this need, we have developed a tool for the Generation of Automatic Neuron Graph-Like Interconnected Arrangements (GANGLIA), which automatically generates micro-patterns using graph drawing algorithms to place the cells based on an input array of neuron connections and generate micro-patterns in a variety of common file formats. Four network connectivities, ranging in levels of complexity, were used to assess GANGLIA's performance. As the complexity increased, the number of intersections of neurite paths and the amount of time GANGLIA took to generate the pattern increased. However, for the most complex circuit tested here, GANGLIA took less than 8 *s* to generate a micro-pattern, which is faster than manually generating an equivalent model. The fast, automatic generation of micro-patterns has the potential to support the design and fabrication process of complex neuron circuits *in vitro* for biohybrid control.

Keywords: neuron · graph drawing · biohybrid controllers · circuit

1 Introduction

Biohybrid controllers incorporate live biological neurons to process information and generate signals to control a periphery. Biological neural controllers for biohybrid robots have been fabricated using a variety of methods, including directly using intact networks, such as intact brains in live animals [6], spinal explants [17], and intact neuromuscular tissues [39]. Alternatively, cultured neurons can be used for biohybrid control, either on a multi-electrode array to control a simulated animal [9] or a robot [18,29,38] or as neurospheres co-cultured with

muscle actuators [2,3]. Although these studies made several advances to implement neural controls *in vitro* for biohybrid systems, the neurons stochastically self-assembled into circuit configurations. Not only does this make the resulting controller difficult to replicate, but this does not enable researchers to prescribe specific circuit configurations or control dynamics. Techniques and tools are needed to design and optimize a controller for specific functionalities and fabricate controllers with custom and repeatable circuit connectivity.

To control the network connectivity of neurons *in vitro*, microfabrication techniques can be used to control the placement and growth directions of neurites. One common technique is to use microcontact printing to constrain cell growth. Microcontact printing takes advantage of the neurons' natural chemical and mechanical sensitivity to control neuron polarization and guide network formation, by either using a gradient pattern of proteins [28] or patterning areas with cytophilic proteins on a cytophobic surface [24,36,41]. Another technique uses microstructures and microfluidics to limit where neurons can grow physically. This can allow for the isolation and placement of individual cells using optimized channel geometries [15,37], microsieves [25], or microwells [27,35]. Specialized designs of the channel geometry also enabled the control of regions that selectively allow for only neurite outgrowth using channels with bottlenecks [21] or narrow, straight microgrooves [26,30,34]. Using similar microfabrication techniques could enable researchers to produce biohybrid controllers with reproducible biological neuronal networks. Although these techniques already demonstrate the capability to guide the formation of simple neuronal circuits between cells [24,41] or specific populations of cells [21,30,34], manually designing and generating pattern layouts to represent more complex circuits, becomes increasingly difficult and time-consuming as network complexity increases, particularly if circuits must be designed manually.

Current existing neuron network modeling tools, such as NEURON [5], Brian 2 [32], and NEST [14], focus on the network and cellular dynamics, rather than producing network layouts for *in vitro* fabrication. Other visualization tools, such as BlenderNEURON [4], are suited for creating reconstructions of biological neurons and circuits akin to those in the brain, which would be challenging to reproduce using current *in vitro* constrained neuron circuit microfabrication techniques. Furthermore, the manual editing of each neuron's morphology to create specific circuits will also suffer from challenges similar to those encountered in the manual design of microfabrication pattern layouts for complex circuits. Therefore, to bridge the gap between *in vitro* constrained neuron culture techniques and producing specific complex circuits, a tool for automatically generating circuit layouts using existing experimental techniques is needed.

To supplement the existing microfabrication techniques towards reproducible, customized biohybrid controllers, we present a new 2D neuron circuit layout tool for the Generation of Automatic Neuron Graph-Like Interconnected Arrangements (GANGLIA) to create micro-patterns from a list of neuron connections automatically. The automatic generation of patterns can greatly reduce the difficulty and time required to design viable stamps and scaffolds, especially for

complex circuits. For neuron circuits to be achievable *in vitro*, the placement of each soma must be distinct, and paths for each neurite cannot overlap to control the network connectivity. Similar challenges are addressed when generating visualizations for complex graphs in discrete mathematics, particularly in the problem of crossing minimization and uniform distribution of vertices [11,33]. Thus, GANGLIA uses graph drawing algorithms to determine candidate locations for neuron somas and neurites to rapidly produce patterns in seconds based on the desired circuit connectivity. The entire micro-pattern can be exported to common model files, including STEP, DXF, and SVG. These files can be used either to fabricate a 3D-printed scaffold for microfluidic chips or for microcontact printing stamps created using soft lithography.

2 Methods

2.1 Micro-pattern generation algorithm

To automatically design micro-patterns for custom biological neuron network circuitry, a pipeline for GANGLIA was developed in Python 3.9.7 (https://www.python.org/). GANGLIA takes an input of neuron connections, paired in an array format, and determines candidate soma and branching node locations for the micro-pattern using either the Fruchterman-Reingold [7,13] or Kamada-Kawai [7,16] graph drawing algorithms from the iGraph (0.9.11) Python library (https://python.igraph.org/) [7] (Refer to Algorithm 1). Then, GANGLIA parametrically generates a micro-pattern for the circuit using CadQuery (2.2.0) (https://cadquery.readthedocs.io/). CadQuery enables the micro-pattern to be exported to several formats (STEP, DXF, SVG, PNG), which can then be used in external software to physically create the micro-pattern using other methods, such as 3D printing (STEP file) or microcontact printing (DXF, SVG).

By implementing Algorithm 1, GANGLIA generates two potential layouts for the placement of the somas using the Kamada-Kawai [7,16] and Fruchterman-Reingold [7,13] graph drawing algorithms (Figure 1). After the user selects the

Algorithm 1 Determine candidate soma and branching node locations

Input: Neuron Connectivity: $C = (N_{pre,1}, N_{post,1}) \dots (N_{pre,i}, N_{post,i})$
Output: Soma Locations: $(x_{N_1}, y_{N_1}) \dots \left(x_{N_j}, y_{N_j}\right)$

for Each Neuron j **do**
  if N_j has > 1 post-synaptic connections **then**
    Append C to insert a branching node.
  end if
end for
Input connection list into the graph drawing algorithms
Display intermediate visual output from graph algorithm
User input on which graph drawing result to use $\rightarrow (x_{N_1}, y_{N_1}) \dots \left(x_{N_j}, y_{N_j}\right)$

Algorithm 2 Generate neural circuit micro-patterns

Inputs: Neuron Connectivity: $C = (N_{pre,1}, N_{post,1}) \dots (N_{pre,i}, N_{post,i})$
Soma Locations: $(x_{N_1}, y_{N_1}) \dots \left(x_{N_j}, y_{N_j}\right)$
Outputs: Neural Circuit Micro-Patterns (STEP, DXF, SVG, PNG)

```
Adjust soma and branching nodes positions based on scaling factor
for Each connection pair i do
    if Cells are mutually connected without connections to other cells then
        Add a curved path connecting the two cells
        Add a gap to separate the axonal path from the dendritic path
    else if The pre-synaptic cell connects to a branching node then
        Add a long, straight path between branching node and pre-synaptic cell
    else
        Add a short path from post-synaptic cell towards pre-synaptic cell
        Add a long path from pre-synaptic cell towards post-synaptic cell
        Leave a short gap between axonal path and dendritic path
    end if
end for
for Each Neuron j do
    a_j ← Number of Connections for N_j
    if a_j < 4 then
        if N_j does not have an axon then
            Add one long path for the axon
            Add (4 − a_j − 1) short paths for dendrites
        else
            Add (4 − a_j) short paths for dendrites
        end if
    end if
    Add circle for soma at (x_{N_j}, y_{N_j})
end for
Output neural circuit micro-patterns as desired file type
```

desired layout, Algorithm 2 generates a micro-pattern. Representations of the micro-pattern can be exported into several file types for 3D-printable CAD models (STEP file) and 2D representations (DXF, SVG, PNG) (Figure 1).

2.2 Performance assessment of GANGLIA

To test the functionality of GANGLIA, micro-patterns were generated for four different connectivity networks: (1) a half-center oscillator inspired by central pattern generators [22], (2) a human-generated network composed of 9 cells, (3) a network for the control of a single limb joint for rat locomotion [10], and (4) a Boolean network for the control of *Aplysia* feeding [40]. These networks varied in their level of complexity, as determined by their number of cells and cell-to-cell connections. For each circuit, after determining the soma and branching node locations using the graph drawing algorithm, the circuit pattern is generated and scaled based on the desired lengths for the dendritic and axonal paths. To scale the pattern, the average distance of each connection was calculated based on

the initial soma and branching node locations. The average distance was used to scale the final pattern size relative to a target length between the center of two somas. The target length is the sum of the following: target axon length, dendrite length, gap distance between the axon and the dendrite ("synaptic cleft"), and the soma diameter. This ratio between the target length and the average distance was used to scale the initial soma and branching node positions. After scaling the positions, the neurite pathways for each connection were generated (Algorithm 2). The dendrite length and gap distance were kept constant, but the axon length was variable for each cell, depending on the final distance between the two connected cells (See Algorithm 2). Each neurite connection must have distinct pathways between the pre- and post-synaptic cell that do not intersect with other pathways to pattern these networks *in vitro* using microcontact printed stamps or microfluidic chips. Thus, to assess GANGLIA's performance in generating micro-patterns for these networks, the number of intersections between neurite pathways and the computational run time were measured for each pattern generated.

To measure the computational run time, the tool was used on a Windows 10 machine with an AMD Ryzen 7 3700X 8-core processor, 16 GB RAM, and an NVIDIA GeForce RTX 2080 SUPER graphics card. The tool was run in the Anaconda 3 distribution of Spyder 5.4.2. The run-time calculated included the time the tool took to complete the two procedures in Algorithms 1 and 2 but excluded the time in which the tool waited for any user inputs and the time the tool took to export the files.

3 Results and Discussion

GANGLIA automatically generated micro-patterns for all four connectivity networks used as test cases (Figure 2). Since the automatic pattern generation is parametrically driven, different elements of the network, such as the area for the soma and the lengths of the axons and dendrites, can be used to scale the pattern to a larger or smaller size. The area designed for the soma can easily be scaled to either support smaller single-cell bodies or larger neurospheres. Furthermore, the lengths of the neurites can be either user-defined or potentially driven by realistic sizes based on experimental studies of neuron development [12,19,20]. However, with thicker neurite widths, there is an increased risk of overlaps between distinct neurite paths.

The number of intersections also increased with network complexity, as characterized by the number of cells, branching nodes, and connections (Table 1). Overlapping neurite paths is not desirable since these would not allow for the control of the connectivity between cells. Thus, these points of intersection could indicate locations where the layout of the somas and branching nodes may need to be modified to make distinct neurite paths. One potential option could be including additional graph drawing algorithms, such as the Davidson-Harel layout [7,8], the distributed recursive graph layout (DrL) [7,23], or the large graph layout (LGL) [1,7], to provide more options for soma placements that might

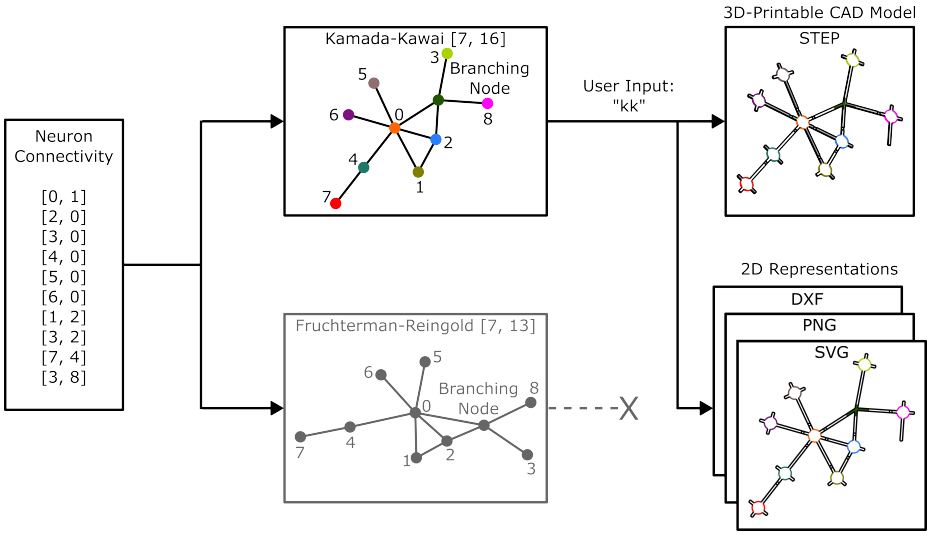

Fig. 1. Using only a list of paired values, indicating which two neurons are connected, GANGLIA can generate a micro-pattern for visually representing the network. Currently, GANGLIA provides the user with two possible layout options; each determined using a different graph drawing algorithm (Fruchterman-Reingold [7,13] or Kamada-Kawai [7,16]). Once the user inputs the desired layout (in this figure, Kamada-Kawai or "kk" was selected, as indicated by the continuing solid black arrows), GANGLIA can finalize the micro-pattern design and export the design in four file types: STEP, DXF, SVG, and PNG. The dashed line from the Fruchterman-Reingold result indicates that the user did not select that layout.

not include these intersections. Another alternative could be scaling GANGLIA to use a three-dimensional space, rather than only creating planar patterns, to accommodate increasingly complex patterns. Several graph drawing algorithms have already been implemented as part of the iGraph library, such as the 3D Fruchterman-Reingold [7,13], 3D Kamada-Kawai [7,16], and 3D DrL [7,23]. Furthermore, complex circuits could potentially be split into smaller, simpler subcircuits with 2D layouts, which could be combined to form a 3D network. However, when translating these designs to physical stamps or scaffolds, creating a 3D, user-defined circuit *in vitro* may require more complex cell manipulation techniques. Furthermore, experimental validation for fabricating these networks *in vitro* by verifying expected synaptic connections and circuit activity is needed.

In addition to the number of intersections, the time to generate a micro-pattern also increased with complexity (Table 1). The difference between the time to generate a pattern using either graph drawing algorithm was small since most of the computational time was spent to generate the CAD model (Algorithm 2, Table 1). Despite the increase in run time, GANGLIA still only takes seconds to generate a full 3D-printable CAD model for the micro-pattern, which could take a human designer several minutes to hours to generate manually.

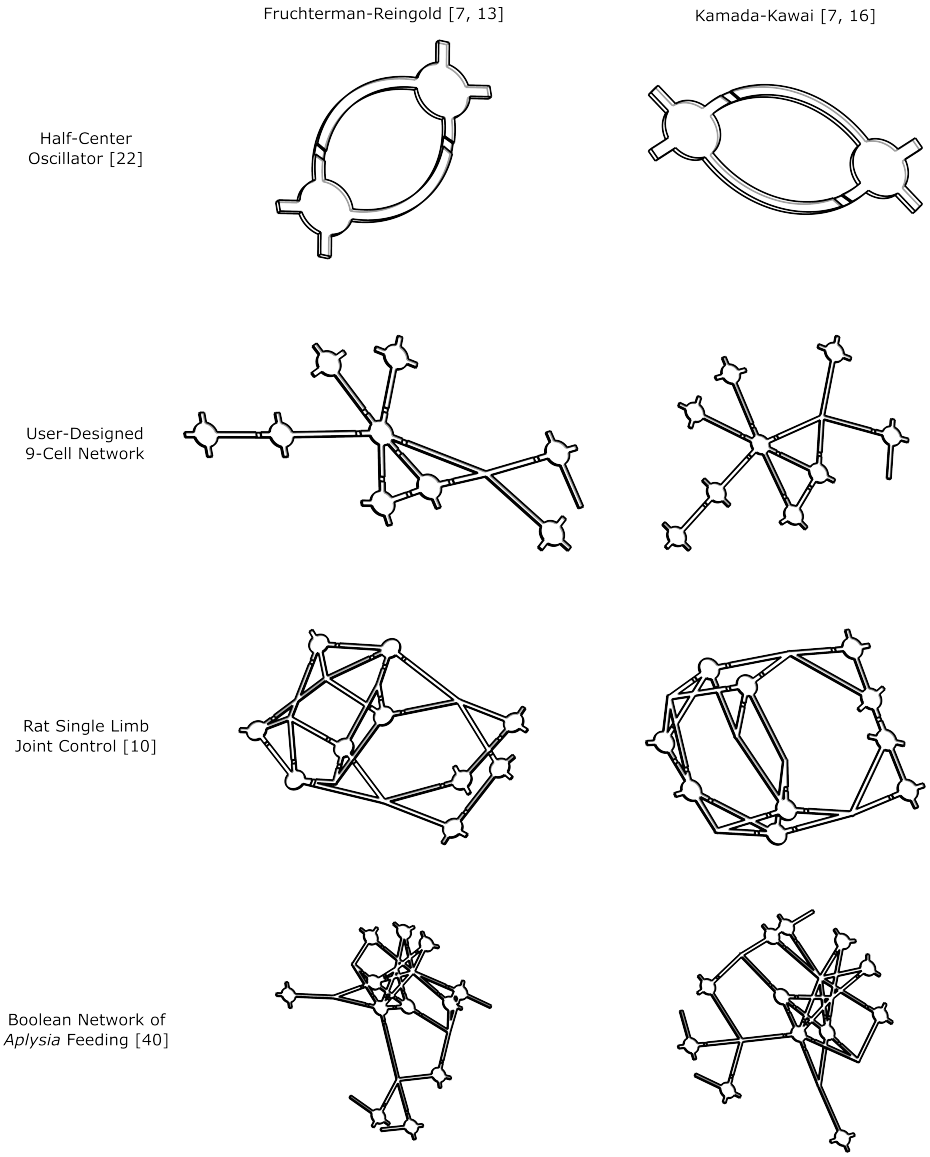

Fig. 2. Four different networks (half-center oscillator [22], user-generated 9-cell network, rat single limb joint control [10], and Boolean network of *Aplysia* feeding [40]) were used to test GANGLIA's micro-pattern generation capabilities. For each network, GANGLIA generated distinct layouts using different graph drawing algorithms (Fruchterman-Reingold [7,13] or Kamada-Kawai [7,16]

Table 1. The run time for each procedure described in Algorithms 1 and 2 automatically create a 3D-printable CAD model for a neuron network micro-pattern and the number of resulting neurite path intersections correspond to the complexity of the network, as characterized by the number of cells, branching nodes, and synapses (cell-to-cell connections). The first procedure (Algorithm 1) determine candidate positions and the second procedure (Algorithm 2) generate the micro-pattern are indicated by $p1$ and $p2$, respectively.

Network	Cells	Nodes	Synapses	Algorithm	Intersections	Run Time (s) $p1$	$p2$
Half-Center Oscillator [22]	2	0	2	FR	0	0.178	0.278
				KK	0	0.178	0.275
User-Designed 9-Cell Network	9	1	10	FR	0	0.207	2.304
				KK	0	0.205	2.322
Rat Single Limb Joint Control [10]	10	6	20	FR	4	0.241	4.831
				KK	9	0.238	5.200
Boolean Network of *Aplysia* Feeding [40]	12	6	22	FR	9	0.241	7.252
				KK	9	0.242	7.148

4 Conclusion

Biohybrid controllers using biological neurons have demonstrated the ability to control different peripheries but need to employ fabrication methods that are repeatable and enable a user to implement a designed circuit scheme. Current microfabrication and micromanipulation techniques enable researchers to control neuron cell placement and circuit formation when fabricating biohybrid controllers. However, existing techniques require the user to manually design the circuit schematic, which becomes increasingly difficult and time-consuming with increasingly more complex network requirements. To supplement existing microfabrication tools for creating user-designed circuits of biological neurons *in vitro*, GANGLIA was developed to automatically generate a micro-pattern using an input of paired neuron connections and graph drawing algorithms. To assess the performance of GANGLIA's pattern generation, four network connectivities, with varying complexity, were used: a half-center oscillator [22], a human-designed 9-cell network, a control network for a single rat limb joint [10], and a control network for *Aplysia* feeding [40]. With the increase in complexity, the number of intersections and the computational run time increased. However, the run time for the most complex network (*Aplysia* feeding) was far less than the time required to generate an equivalent model manually. In the future, GANGLIA could be further extended into 3D design space, which may reduce

or eliminate the number of intersections. Additionally, patterns generated by GANGLIA could be coupled with neuron growth simulations [31] to predict how networks will form and function when grown on resulting patterns. Overall, the automatic generation of micro-patterns for more complex neuron circuits provided by GANGLIA will greatly reduce circuit prototyping design cycle time and supports the future creation *in vitro* biohybrid controllers.

Code Availability The designs and the pattern-generation tool GANGLIA presented in this paper are available at https://doi.org/10.5281/zenodo.7790394.

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