



# NETWORK NEURO SCIENCE

an open access  journal



Citation: Mehta, K., Goldin, R. F., Marchette, D., Vogelstein, J. T., Priebe, C. E., & Ascoli, G. A. (2021). Neuronal classification from network connectivity via adjacency spectral embedding. *Network Neuroscience*, 5(3), 689–710. [https://doi.org/10.1162/netn\\_a\\_00195](https://doi.org/10.1162/netn_a_00195)

DOI:  
[https://doi.org/10.1162/netn\\_a\\_00195](https://doi.org/10.1162/netn_a_00195)

Supporting Information:  
[https://doi.org/10.1162/netn\\_a\\_00195](https://doi.org/10.1162/netn_a_00195)

Received: 17 December 2020  
Accepted: 2 April 2021

Corresponding Author:  
Giorgio A. Ascoli  
[ascoli@gmu.edu](mailto:ascoli@gmu.edu)

Handling Editor:  
Olaf Sporns

Copyright: © 2021  
Massachusetts Institute of Technology  
Published under a Creative Commons  
Attribution 4.0 International  
(CC BY 4.0) license



## METHODS

# Neuronal classification from network connectivity via adjacency spectral embedding

Ketan Mehta<sup>1\*</sup>, Rebecca F. Goldin<sup>2\*</sup>, David Marchette<sup>3</sup>,  
Joshua T. Vogelstein<sup>4</sup>, Carey E. Priebe<sup>4</sup>, and Giorgio A. Ascoli<sup>1</sup>

<sup>1</sup>Department of Bioengineering and Center for Neural Informatics, Structures, and Plasticity, George Mason University, Fairfax, VA, USA

<sup>2</sup>Department of Mathematical Sciences and Center for Neural Informatics, Structures, and Plasticity, George Mason University, Fairfax, VA, USA

<sup>3</sup>Naval Surface Warfare Center, Dahlgren, VA, USA

<sup>4</sup>Department of Applied Mathematics and Statistics, Johns Hopkins University, Baltimore, MD, USA

\*These authors contributed equally to this work.

**Keywords:** Directed graphs, Expectation maximization, Neural circuits, Stochastic block models, Cell-type classification, Spectral embedding

## ABSTRACT

This work presents a novel strategy for classifying neurons, represented by nodes of a directed graph, based on their circuitry (edge connectivity). We assume a stochastic block model (SBM) in which neurons belong together if they connect to neurons of other groups according to the same probability distributions. Following adjacency spectral embedding of the SBM graph, we derive the number of classes and assign each neuron to a class with a Gaussian mixture model-based expectation maximization (EM) clustering algorithm. To improve accuracy, we introduce a simple variation using random hierarchical agglomerative clustering to initialize the EM algorithm and picking the best solution over multiple EM restarts. We test this procedure on a large ( $\approx 2^{12}$ – $2^{15}$  neurons), sparse, biologically inspired connectome with eight neuron classes. The simulation results demonstrate that the proposed approach is broadly stable to the choice of embedding dimension, and scales extremely well as the number of neurons in the network increases. Clustering accuracy is robust to variations in model parameters and highly tolerant to simulated experimental noise, achieving perfect classifications with up to 40% of swapped edges. Thus, this approach may be useful to analyze and interpret large-scale brain connectomics data in terms of underlying cellular components.

## INTRODUCTION

A functionally relevant, quantitative description of cellular diversity in the brain remains a pressing open problem in neuroscience. Traditionally, investigators have classified neurons by subsets of multifarious properties, including physiology, biochemistry, and morphology (e.g., a fast-spiking, parvalbumin-expressing, aspiny interneuron). In spite of the widespread and foundational use of the notion of cell class, there is no formal definition of this concept, and how exactly a cell class relates to network connectivity remains a matter of considerable debate in the community (DeFelipe et al., 2013; Petilla Interneuron Nomenclature Group et al., 2008). In particular, given a “solved” connectome (a complete list of all neurons and

their connections), is it possible to objectively find the number of neuronal connectivity classes, and to assign each neuron to a class? This would also answer the related open question of how many cell classes there are from the connectomics perspective (Hamilton, Shepherd, Martone, & Ascoli, 2012).

In this work we introduce a novel strategy for classifying neurons based on their circuitry. In particular, after formalizing the concept of cell class based on network connectivity, we present a technique to derive the number of cell classes from a neuronal connectome, and to assign each neuron to a class. Using neurobiologically realistic surrogate data, we demonstrate that this technique is robust and efficient.

We begin by asking a mathematical question derived from the neuroscientific one. Recall that a **directed graph**  $(V, E)$  consists of vertices  $V$  (a finite set), and directed edges  $E$ , a subset of ordered pairs of  $V \times V$ . We assume the directed graph is **simple**, that is, there is at most one edge between any two distinct vertices, and no edge from a vertex to itself, though we allow the possibility of edges in either direction. For the purpose of our analysis, each connectome may be represented by such a directed graph, wherein the vertex represents a neuron and the edge represents a directed synaptic (usually axon-dendrite) connection. Further, we adopt a generative model approach by using a stochastic block model (SBM) to add additional structure to the directed graph. In this model vertices are partitioned into nonoverlapping groups called blocks, such that the probability of an edge between two vertices depends only on their respective block memberships. Vertices in the same block are thus stochastically equivalent. Given a directed SBM graph, our goal is then to estimate the number of blocks and assign each vertex to its respective block.

Recently, SBMs have been successfully used to model connectomes (Moyer et al., 2015; Pavlovic, Vértes, Bullmore, Schafer, & Nichols, 2014), as well as to identify network community structures within connectomes (Betzel, Medaglia, & Bassett, 2018; Faskowitz, Yan, Zuo, & Sporns, 2018). Our approach here, however, is different from these studies in two important aspects. First, we use surrogate connectomic data loosely inspired by the entorhinal-CA1 circuit of the rodent hippocampal formation. The scale and structure of the neuronal network analyzed in this work is therefore vastly different, with substantially larger graphs ( $\approx 2^{12}$ – $2^{15}$  vertices) and sparse ( $\approx 4\%$ ) connectivity. Second, and more fundamentally, our focus is on developing a robust mathematical framework using spectral graph clustering to capture the latent block structure of the directed graph. We are motivated by recent results (Priebe et al., 2017, 2019; Sussman, Tang, Fishkind, & Priebe, 2012) that demonstrate the use of adjacency spectral embedding (ASE) in conjunction with Gaussian mixture model (GMM)-based clustering to estimate block membership. Here we adopt and modify the GMM $\circ$ ASE framework, and present a strategy to cluster large, sparse graphs modeled from surrogate connectomic data.

Given a graph, we begin by embedding it into a much lower dimensional space by computing the singular value decomposition of a slightly modified version of the adjacency matrix. Since we consider directed graphs, we embed a concatenation of the left and right singular vectors, which correspond to the outgoing (presynaptic) and incoming (postsynaptic) connections, respectively. Following the embedding, the latent vectors are modeled as a GMM and clustered using the expectation maximization (EM) algorithm. However, the convergence of the EM algorithm is highly sensitive to the starting values chosen to initialize the algorithm, especially for the multivariate GMM case (Biernacki, Celeux, & Govaert, 2003; Kwedlo, 2015; Shireman, Steinley, & Brusco, 2017), and often gets trapped in a local optimum. Therefore, we propose using a multiple restart approach wherein we apply hierarchical agglomerative clustering to randomly initialize and start the EM algorithm multiple times, and subsequently pick the model with the largest value of Bayesian information criterion (BIC) over multiple restarts.

Surrogate data:  
Synthetic data generated using a mathematical model.

Graph:  
A formalization of a network in which the nodes and their interactions are represented as vertices and edges, respectively.

Stochastic:  
The property of being described by a random probability distribution.

Spectral embedding:  
Mapping of a high-dimensional matrix into a relatively low-dimensional space by making use of its spectrum (singular values).

Latent vector:  
A vector of “hidden” variables (often in a lower dimensional space) that capture the underlying properties of the data.

Expectation maximization algorithm:  
An iterative probability density estimation technique to find the best fit of the assumed statistical model to the observed data.

Bayesian information criterion:  
A criterion for model selection that measures the trade-off between model fit and complexity of model.

We perform a series of experimental simulations with surrogate data to validate the effectiveness of the proposed multiple random restart EM. The simulation results demonstrate the proposed clustering strategy to be extremely effective in successfully recovering the true number of classes and individual class assignment of the vertices. The random multiple restart approach also heavily outperforms GMM-based hierarchical partition initialization (Scrucca & Raftery, 2015), while having the advantage of being broadly stable over a wide selection of embedding dimensions, as choosing an optimal value for dimensional embedding remains an open problem with spectral graph clustering in general. The proposed approach is also robust to variations in model parameters and scales extremely well as the number of neurons in the network increases. Moreover, our analysis shows this method to be highly tolerant to noise in the form of edge swaps akin to experimental errors in pre- or postsynaptic neuron identification.

## MODELING THE CONNECTOME

### Stochastic Block Models

Consider a directed graph  $(V, E)$  that consists of vertices  $V$  (a finite set), and directed edges  $E$ , a subset of ordered pairs of  $V \times V$ . We write  $(v, w) \in E$  for  $v, w \in V$  if there is a directed edge from  $v$  to  $w$ . Further, we assume the graph to be **simple**, that is,  $(v, w) \in E$  implies  $v \neq w$ . As  $E$  is a set of ordered pairs, there is at most one directed edge from any vertex  $v$  to a distinct vertex  $w$ . We allow the possibility of edges  $(v, w)$  and  $(w, v)$ . We formally define a partitioned directed graph as follows:

For a vertex set  $V$ , a **block assignment**  $\tau$  is an assignment of a group membership, denoted by an integer  $1, 2, \dots, k$ , to each vertex in  $V$ . Explicitly, for a fixed positive integer  $k \leq |V|$ ,

$$\tau : V \rightarrow \{1, 2, \dots, k\},$$

where  $|V|$  is the size of the vertex set. A block assignment associates a **class** to each vertex  $v$ , indicated by the value  $\tau(v)$ . In particular, two vertices are in the same class if and only if they have identical values under  $\tau$ . We formally define a partitioned directed graph as follows:

**Definition 1.** A **partitioned directed graph** is a triple  $(V, E, \tau)$ , where  $(V, E)$  is a simple directed graph and  $\tau : V \rightarrow \{1, \dots, k\}$  is a block assignment that partitions the vertices into  $k \leq |V|$  disjoint (nonoverlapping) subsets

$$V_j := \{v \in V : \tau(v) = j\}, \quad \text{for } j = 1, \dots, k.$$

The set  $V_j$  consists of vertices in class  $j$ .

Our functional assumption here is that the structural connectome can be represented as a graph with unweighted (binary) edges, that is, a synaptic connection is either present or absent. Further, we assume that the probability of a pre- to postsynaptic connection from neuron  $v$  to neuron  $w$  depends solely on the classes  $\tau(v)$  and  $\tau(w)$ . This is well modeled by a stochastic block model (Holland, Laskey, & Leinhardt, 1983; Holland & Leinhardt, 1981), in which stochastically equivalent vertices are partitioned together into classes. In particular, a SBM assumes that edges between vertices from the  $i$ th class to those in the  $j$ th class can be modeled as independent Bernoulli trials with parameter  $p_{ij}$ . Let  $P = (p_{ij})$  be a matrix collecting these parameters. We then formally define the generative model of the standard directed SBM as follows.

**Definition 2.** A **directed stochastic block model** is a generative model for directed graphs. Let  $n$  be the number of nodes (vertices),  $k$  the number of groups (classes),  $P = (p_{ij}) \in [0, 1]^{k \times k}$  the block connectivity probability matrix (edge probabilities), and  $\tau : V \rightarrow \{1, \dots, k\}$  the assignment

Bernoulli trials:  
Independent random experiments,  
each with exactly two possible  
outcomes occurring with  
probabilities  $p$  and  $1 - p$ ,  
respectively.

of each node to a group. A directed SBM graph is a partitioned directed graph  $G = (V, E, \tau)$  whose edges are independent Bernoulli draws with probability  $P\{(v, w) \in E\} = p_{\tau(v), \tau(w)}$ .

Let  $\rho_j := |V_j|/n$  be the proportion of vertices in the  $j$ th group. The  $k$ -tuple  $\rho := (\rho_1, \dots, \rho_k)$  indicates the proportional sizes of these classes. Note that  $\{V_1, \dots, V_k\}$  and  $\rho$  depend only on  $\tau$ .

In a general SBM (Abbe, 2017) (often referred to simply as a SBM, such as in Sussman et al., 2012) the vertex assignment, and thus the class size  $|V_j|$  of the generated graph, is subject to a random process. However, in our generative model the assignment is instead specified by the block assignment function  $\tau$ . While in theory the number of classes is bounded above by the size of the vertex set, most practical implementations of SBM inference (Abbe, 2017; Funke & Becker, 2019; McDaid, Murphy, Friel, & Hurley, 2013) constrain  $k \ll |V|$ . This constraint allows for successful prediction of the block assignments using the limited vertex set size, as well as, in our case, a meaningful resulting neuronal classification.

### Connectome Generation

The experimental design begins with using a directed SBM to generate stochastic realizations (simulations) of the biological connectome. The surrogate model used is loosely inspired by the entorhinal-CA1 circuit of the rodent hippocampal formation based on Hippocampome.org data (Wheeler et al., 2015). Specifically, we consider a directed neuronal network consisting of  $n$  cells, where  $n$  varies, and  $k = 8$  distinct cell types. Each cell type is briefly described in Table 1. The model is parametrized by the connectivity probability matrix

$$P = \begin{pmatrix} .02 & .02 & .006666667 & .00 & .02 & .04 & .04 & .02 \\ .02 & .00 & .006666667 & .02 & .00 & .00 & .00 & .00 \\ .02 & .00 & .006666667 & .00 & .00 & .00 & .00 & .00 \\ .02 & .00 & .006666667 & .02 & .00 & .00 & .00 & .00 \\ .02 & .02 & .006666667 & .00 & .02 & .00 & .00 & .00 \\ .00 & .00 & .00 & .00 & .00 & .04 & .04 & .02 \\ .04 & .00 & .013333333 & .04 & .00 & .02 & .02 & .01 \\ .00 & .00 & .00 & .00 & .00 & .02 & .02 & .01 \end{pmatrix}, \quad (1)$$

and a block membership vector  $\rho$  that denotes the proportions of the cells (vertices) assigned each cell type (class),

$$\rho = (0.48120, 0.12207, 0.03052, 0.09155, 0.06104, 0.07629, 0.07629, 0.06104). \quad (2)$$

We chose the specific values of  $P$  as rounding approximations of recently published experimental data derived from the measured lengths of spatially overlapping presynaptic axons and postsynaptic dendrites from the indicated neuron types in the appropriate anatomical volumes (Tecuatl, Wheeler, Sutton, & Ascoli, 2020). Furthermore, we selected the proportions of neurons in each type defined in the individual components of  $\rho$  based on estimates obtained by numerical optimization of evidence sourced from Hippocampome.org using a recently introduced operations research approach (Attili, Mackesey, & Ascoli, 2020). The assignment  $\tau$  of cells to cell types simply maps the first  $n\rho_1$  cells to the first type, then next  $n\rho_2$  cells to the second type, and so on.

Partitioned directed graphs are generated using SBM, with the vertices proportioned into blocks according to  $\rho$  (2), and edges drawn as per the block probabilities specified in  $P$  (1). We label the vertices of  $V$  by  $v_1, \dots, v_n$ . Each directed graph is uniquely associated with an **adjacency matrix**  $A$ , an  $n \times n$  binary matrix with the  $l$ th entry given by 1 if  $(v_\ell, v_m) \in E$  and 0 otherwise.

**Table 1.** The eight cell classes

|                                     |  |
|-------------------------------------|--|
| CA1 Pyramidal                       | Principal output neurons of the hippocampus. One of the most studied and best characterized excitory neurons of the mammalian brain.   |
| CA1 Oriens/Lacunosum-Moleculare     | Local inhibitory neurons. Dendrites are in the oriens layer and axons start in the oriens and go up to lacunosum-moleculare.   |
| CA1 Basket                          | Local peri-somatic inhibitory interneurons. Axons target pyramidal and basket cells. Dendrites span all layers of CA1.   |
| CA1 Perforant Pathway-Associated    | Local inhibitory interneurons with axons and dendrites confined to the lacunosum-moleculare layer.   |
| CA1 Oriens                          | Local inhibitory interneurons with dendrites and axons confined to the oriens layer.   |
| Entorhinal Cortex Layer 5 Pyramidal | Deep layer excitatory neurons with dendrites and axons extending through the deep and superficial layers of the entorhinal cortex.   |
| Entorhinal Cortex Layer 3 Pyramidal | Superficial layer excitatory neurons. Dendrites span through the deep and superficial layers of the entorhinal cortex; axons start in layer 3 and project to CA1 lacunosum-moleculare. |
| Entorhinal Cortex GABAergic Cells   | Inhibitory local interneurons with axons and dendrites through the deep and superficial layers of the entorhinal cortex.   |

### ADJACENCY SPECTRAL EMBEDDING

Given an  $n \times n$  adjacency matrix  $A$  generated by a directed SBM, the goal is to predict the number of classes and recover the class assignment for each individual vertex of the graph, with no prior knowledge of  $k$ ,  $P$ , or  $\rho$ . The first step is to embed the adjacency matrix into a lower dimensional Euclidean space via singular value decomposition.

#### Singular Value Decomposition

Any real valued matrix  $A$  may be decomposed into a product  $A = UDV^t$ , where  $D$  is a diagonal matrix with nonnegative real entries, and  $U$  and  $V$  are real valued orthogonal matrices, called a **singular value decomposition**. We may choose  $D$  so that its entries, called the **singular values**, are nonnegative and weakly decreasing, in which case  $D$  is uniquely determined by  $A$ . The columns of  $U$  and  $V$  are called **singular vectors**.

In contrast,  $U$  and  $V$  are not unique; if the entries of  $D$  are distinct and nonzero, then  $U$  and  $V$  are determined up to a simultaneous factor of  $\pm 1$  in each column of  $U$  and  $V$ . If there are repeating nonzero entries of  $D$ , the corresponding singular vectors span a subspace of dimension given by the number of copies of the repeated singular value. Any set of orthonormal vectors spanning this subspace can be used as the singular vectors in  $U$ , with a resulting choice in  $V$ . If any singular values vanish, the corresponding singular vectors in  $U$  and  $V$  may be chosen independently.

For any  $d \leq \text{rank}(A)$ , one can approximate  $A$  by a rank  $d$  decomposition

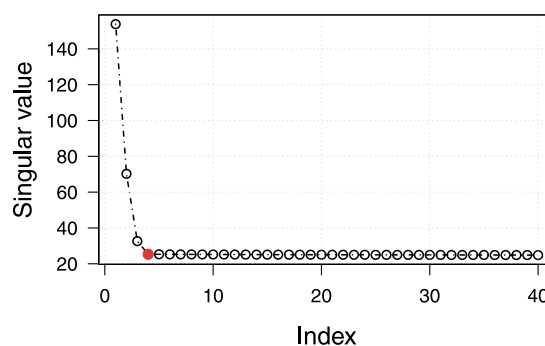
$$A \sim U_d D_d V_d^t,$$

in which  $U_d$  and  $V_d$  are  $n \times d$  matrices, and  $D_d$  is a  $d \times d$  diagonal matrix with nonnegative entries. Let  $X := U_d \sqrt{D_d}$  and  $Y := V_d \sqrt{D_d}$ , so that  $A \sim XY^t$ .

### Embedding in a Lower Dimension

We use a singular value decomposition of a slight perturbation of the adjacency matrix to capture the most salient data in a low-dimensional space. Since we only consider simple graphs with no self edges, all diagonal entries of the adjacency matrix are zero. It has been shown (Marchette, Priebe, & Coppersmith, 2011; Scheinerman & Tucker, 2010) for undirected graphs that artificially augmenting the diagonal with imputed values may improve the embedding in certain cases, in turn leading to fewer misassignments. While similar results have not been proven for the case of directed graphs, we nevertheless modify the adjacency matrix by replacing the diagonal entries via  $A_{ii} = \text{deg}^+(v_i)/(n - 1)$ , where  $\text{deg}^+(v_i)$  is the outgoing degree of the  $i$ th vertex,  $v_i \in V$ . The outgoing degree of the  $i$ th vertex is the number of outgoing edges incident to the vertex, and is calculated by simply summing up all entries of the  $i$ th row of  $A$ . However, since in general for large, sparse graphs  $\text{deg}^+(v_i) \ll n$ , this change in diagonal value has only a small impact on the matrix decomposition. For each directed graph  $(V, E)$  and choice of embedding dimension  $d$ , the vectors forming the columns in the augmented matrix  $\mathbf{X} := [X|Y]^t$  provide a **dot product embedding** of  $A$  in a  $2d$ -dimensional space. The columns of the concatenated matrix  $\mathbf{X}$  are called **latent vectors**.

The optimal choice of  $d$  is a known open problem in literature, with no consensus on a best strategy. The necessity of selecting an optimum  $d$  is based on the fact that only a subset of the singular values of the high-dimensional data are informative and relevant to the subsequent statistical inference. Choosing a low  $d$  can result in discarding important information, while choosing a higher  $d$  than required not only increases computational cost but can adversely effect clustering performance due to the presence of extraneous variables that contribute towards noise in the data. For SBM graphs with large  $n$ , it has been shown (Fishkind, Sussman, Tang, Vogelstein, & Priebe, 2013) that the optimal choice of  $d$  is the rank of the block connectivity matrix  $P$ , however in our context we assume no prior knowledge of  $P$ . A general methodology to choose the optimum value for  $d$  is then to examine the scree plot, the plot of the singular values in weakly decreasing order, and look for an “elbow point” that determines the cutoff between relevant and nonrelevant dimensions based on the magnitude of the singular value. The scree plot for a SBM graph generated using the parameters of our surrogate model (1), (2) is shown in Figure 1. Estimating the elbow point using the unit-invariant knee method (Christopoulos, 2016) yields an optimum value of  $d = 4$ . This choice of  $d = 4$  is also consistent if we instead use an alternative method (Satopaa, Albrecht, Irwin, & Raghavan, 2011) of estimating the distance from each point in the scree plot to a line joining the first and last points of the plot, and then selecting the elbow point where this distance is the largest.

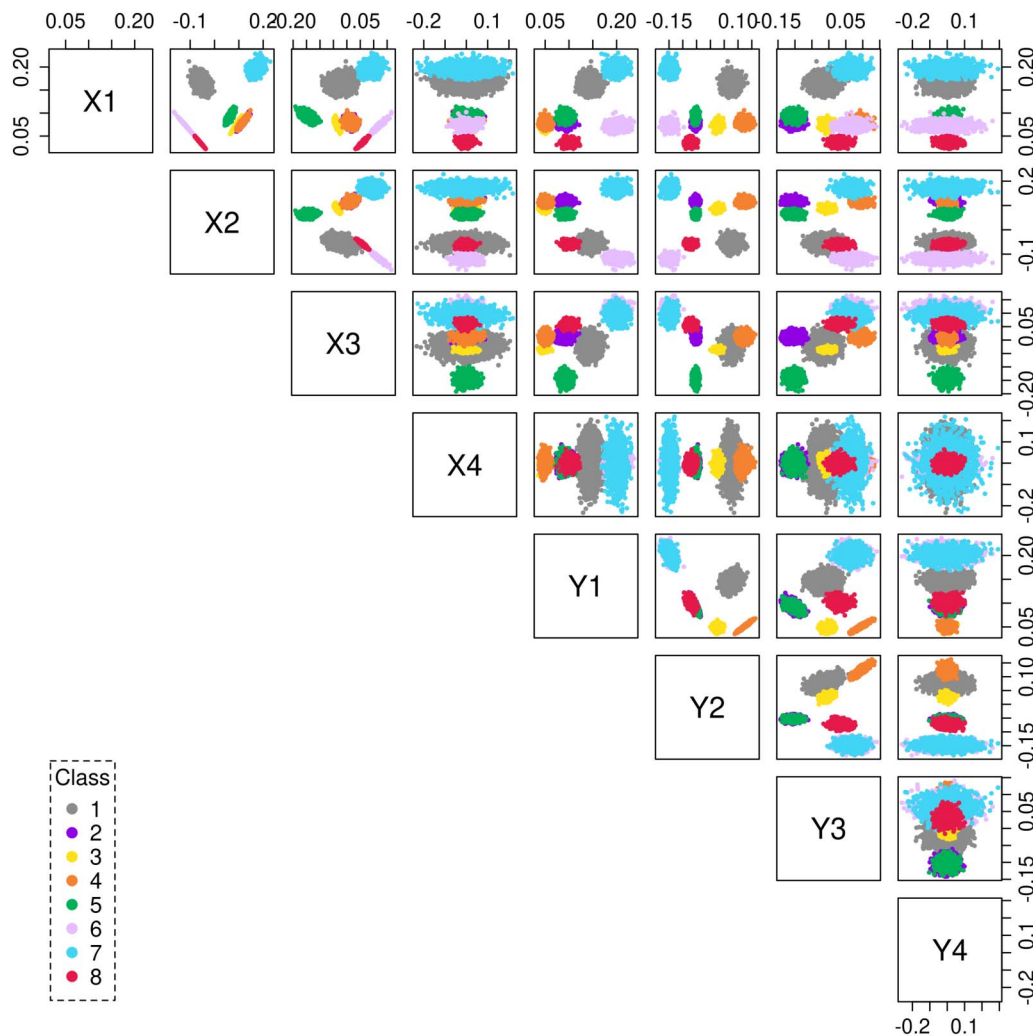


**Figure 1.** Model selection:  $d = 4$  based on the elbow point of the scree plot of singular values ( $n = 16,384$ ). The top  $d$  singular values and their associated left- and right-singular vectors are concatenated to embed the graph in  $\mathbb{R}^{2d}$ .

We apply singular value decomposition directly to  $A$  before clustering, rather than to its Laplacian. For the case of a symmetric  $A$  (undirected graphs), under certain assumptions (Sussman et al., 2012), clustering of the resulting singular value decomposition converges to a negligible number of misclassified vertices. Such results have also been found in similar work applied to the Laplacian (Rohe, Chatterjee, & Yu, 2011; Vogelstein et al., 2019). However, to the best of our knowledge, analogous results for directed graphs have not been explored.

### GAUSSIAN MIXTURE MODEL-BASED CLUSTERING

Let  $A$  be an  $n \times n$  adjacency matrix and  $A \sim XY^t$  be a singular decomposition with  $d$ -singular values. We denote by  $\mathbf{X} = (\underline{x}_1, \underline{x}_2, \dots, \underline{x}_n)^t$  the data (latent vectors) obtained from this decomposition of  $A$ , where  $\underline{x}_i \in \mathbb{R}^{2d}$  denotes the concatenation of the  $i$ th row of  $X$  followed by the  $i$ th row of  $Y$ . Figure 2 shows a scatterplot matrix of the latent vectors distributed in  $\mathbb{R}^{2d}$ , for the choice of embedding  $d = 4$ . The scatterplot depicts the data projected as points onto a two-dimensional subspace, whose coordinates are composed of a pair of the orthogonal singular



**Figure 2.** Scatterplot matrix showing the latent vectors of a SBM graph with  $k = 8$  classes embedded in  $2d = 8$  dimensions. Each data point ( $n = 16,384$ ) is color coded as per its original class assignment.

vectors. The colors represent the original class assignment associated with each data point. The SBM graph was generated using the surrogate model (1), (2) for  $k = 8$  classes, and  $n = 16,384$ .

**Expectation Maximization (EM) Algorithm**

We cluster the data by modeling the latent vectors as a multivariate Gaussian mixture model (GMM) in order to predict the number of components, and the SBM block partition function. For sufficiently dense graphs, and large  $n$ , the adjacency spectral embedding (ASE) central limit theorem demonstrates that  $\underline{x}_i$  behaves approximately as a random sample from a  $k$ -component GMM (Athreya et al., 2016).

Let  $f_j(\underline{x}) = \pi_j \phi(\underline{x}; \underline{\mu}_j, \Sigma_j)$ , where  $\phi(\underline{x}; \underline{\mu}_j, \Sigma_j)$  is the probability density function for the multivariate normal distribution with mean vector  $\underline{\mu}_j \in \mathbb{R}^{2d}$ , covariance matrix  $\Sigma_j$ , and a component weight  $\pi_j$  for  $j = 1, \dots, \kappa$ . The probability density function for the multivariate GMM with  $\kappa \in \mathbb{Z}^+$  components is given by

$$f(\underline{x}_i) = \sum_{j=1}^{\kappa} f_j(\underline{x}_i).$$

The Gaussian mixture model is fitted to the data using the expectation maximization (EM) algorithm. We assume the Gaussian distributions may have aspherical covariances to address clusters in ellipsoidal shapes. The clusters are centered at the mean vector  $\underline{\mu}_j$ , while other geometric features, such as the volume, shape, and orientation, of each cluster are allowed to vary. Assuming the  $n$  data points  $\underline{x}_1, \underline{x}_2, \dots, \underline{x}_n$  are independent draws,

$$f(\mathbf{X}) = \prod_{i=1}^n \sum_{j=1}^{\kappa} \pi_j \phi(\underline{x}_i; \underline{\mu}_j, \Sigma_j).$$

After an initialization of the mixture parameters  $\Theta_{\kappa} = \{\pi_1, \underline{\mu}_1, \Sigma_1, \dots, \pi_{\kappa}, \underline{\mu}_{\kappa}, \Sigma_{\kappa}\}$ , we set

$$\begin{aligned} \tau_{ij} &= \frac{f_j(\underline{x}_i)}{f(\underline{x}_i)} \text{ for } i = 1, \dots, n, & \tau_j &= \frac{1}{n} \sum_{i=1}^n \tau_{ij} = \frac{1}{n} \left( \frac{f_j(\underline{x}_1)}{f(\underline{x}_1)} + \frac{f_j(\underline{x}_2)}{f(\underline{x}_2)} + \dots + \frac{f_j(\underline{x}_n)}{f(\underline{x}_n)} \right) \\ \underline{\mu}_j &= \sum_{i=1}^n \tau_{ij} \underline{x}_i, & \Sigma_j &= \frac{1}{n-1} \sum_{i=1}^n \tau_{ij} (\underline{x}_i - \underline{\mu}_j) (\underline{x}_i - \underline{\mu}_j)^t, \end{aligned}$$

where the product  $(\underline{x}_i - \underline{\mu}_j)(\underline{x}_i - \underline{\mu}_j)^t$  occurring in  $\Sigma_j$  is the tensor (outer) product.

The EM algorithm is used to iteratively improve upon the estimates by maximizing the log-likelihood of the joint probability density function

$$\ell(\mathbf{X}; \Theta_{\kappa}) = \ln f(\mathbf{X}; \Theta_{\kappa}) \tag{3}$$

$$= \sum_{i=1}^n \ln \sum_{j=1}^{\kappa} \pi_j \phi(\underline{x}_i; \underline{\mu}_j, \Sigma_j). \tag{4}$$

We iterate this process until convergence. After the first iteration,  $\sum_j \pi_j = 1$ , and  $\sum_j \tau_{ij} = 1$ . This model assumes that  $\underline{x}_i$  has an associated probability  $\tau_{ij}$  to be in each of the  $j$ th group. Indeed from this description we can define the estimated class assignment as follows: Let  $\tau : V \rightarrow \{1, \dots, k\}$  be given by  $\tau(v_i) = \arg \max_j \tau_{ij}$ .



**Estimating the Number of Clusters**

The model fitting procedure discussed above relies on a given number of GMM components  $\kappa$ , among which to distribute the  $n$  data points. Indeed, assigning each data point to its own cluster ( $\kappa = n$ ) would uniquely identify connectivity behavior of each vertex, but would not illuminate common attributes. At the other extreme,  $\kappa = 1$  provides no distinguishing information among vertices. Let  $\kappa_{\min}$  and  $\kappa_{\max}$  denote the smallest and largest values of practical interest for  $\kappa$ , respectively. We estimate the number of clusters by selecting the value of  $\kappa \in \{\kappa_{\min}, \dots, \kappa_{\max}\}$  that maximizes the Bayesian information criterion. BIC penalizes the model based on the number of free parameters,

$$p_{\kappa} = (\kappa - 1) + 2d\kappa + \left( \binom{2d}{2} + d \right) \kappa, \tag{5}$$

which grows linearly with  $\kappa$  and depends quadratically on the number of singular values  $d$ . Specifically, let  $\hat{\Theta}_{\kappa}$  be the maximum likelihood estimate of the parameters given the data  $\underline{x}_1, \underline{x}_2, \dots, \underline{x}_n$  under the assumption that they are modeled by a multivariate Gaussian mixture model with  $\kappa$  components. The estimated number of classes is defined as

$$\hat{\kappa} = \underset{\kappa_{\min} \leq \kappa \leq \kappa_{\max}}{\operatorname{arg\,max}} \{2\ell(\mathbf{X}; \hat{\Theta}_{\kappa}) - p_{\kappa} \ln(n)\}. \tag{6}$$

For each  $\kappa$ , the GMM fit results in a class assignment  $\hat{\tau}$  of each vector  $\underline{x}_i$  to a group labeled  $\{1, \dots, \kappa\}$ .

**EM Initializations Using Multiple Restarts**

The final parameter estimates of the fitted model are often sensitive to the initial values chosen to start the EM algorithm, especially for the case of finite mixture models (Melnykov & Melnykov, 2012; Shireman et al., 2017). A poor initial choice of the model parameters may cause the EM algorithm to converge to a local but not a global maximum of the likelihood function (Biernacki et al., 2003).

A workaround to the problem of EM initialization is the multiple restart approach (Biernacki et al., 2003; Kwedlo, 2015). Specifically, given a set of data points, the EM algorithm is run  $T$  times (trials), each trial starting with different initial parameters. Each trial is run across all  $\kappa$  values with  $\kappa_{\min} \leq \kappa \leq \kappa_{\max}$ , resulting in  $\hat{\kappa}$ ,  $\hat{\tau}$ , and a maximum BIC value for the trial. The final clustering is selected as the model with the highest BIC across all  $T$  trials. Considering the high prevalence of local maxima in the log-likelihood function, optimal solutions resulting from different trials are typically different. The highest BIC observed across a sufficiently large number of trials corresponds to the best estimate of the global maximum among local optima.

For each trial, an initial estimate of the model parameters is obtained by applying another preliminary clustering to the data. Towards this extent, we compare two variations of agglomerative hierarchical clustering. Inherent advantages of agglomerative hierarchical clustering are that it partitions the data simultaneously into any number of desired clusters, and that, for any trial, the initial clusters are similar across values of  $\kappa$ . In the first method, initial parameters are obtained by partitioning the data using random hierarchical agglomerative clustering (RHAC). In the second approach, initial parameters are obtained by applying model-based hierarchical agglomerative clustering (MBHAC) to a random subset of the data points. Both methods are described in further detail in the following subsections.

**Restarts using random hierarchical agglomerative clustering (RHAC).** At the outset RHAC begins with every data point  $\underline{x}_1, \underline{x}_2, \dots, \underline{x}_n$  in its own cluster. Random pairs of clusters are then

Hierarchical agglomerative clustering:  
A “bottom-up” approach where each observation starts in its own cluster, and pairs of clusters are merged as one moves up the hierarchy.

**Algorithm 1** *mRHEM*<sup>†</sup>

---

**Input:**  $\mathbf{X} = (\underline{x}_1, \underline{x}_2, \dots, \underline{x}_n)^t$

- 1: **Begin** *t*th trial,  $t \in \{1, 2, \dots, T\}$
- 2: Apply RHAC<sup>†</sup> to initialize model parameters  $\Theta_{\kappa}$ ,  $\{\forall \kappa \in \mathbb{Z}^+ : \kappa_{\min} \leq \kappa \leq \kappa_{\max}\}$
- 3: **Loop**  $\kappa \in \{\kappa_{\max}, \kappa_{\max} - 1, \dots, \kappa_{\min} + 1, \kappa_{\min}\}$
- 4: Run EM: iteratively maximizing  $\ell(\mathbf{X}; \Theta_{\kappa})$  until convergence
- 5: **End loop**
- 6:  $\text{BIC}^{(t)} = \max_{\kappa} \{2\ell(\mathbf{X}; \hat{\Theta}_{\kappa}) - p_{\kappa} \ln(n)\}$
- 7: **End trial**
- 8: Select model with highest BIC across all trials,  $\max(\text{BIC}^{(1)}, \text{BIC}^{(2)}, \dots, \text{BIC}^{(T)})$

**Output:** number of classes  $\hat{k}$ , and class assignment  $\hat{\tau}$

---

<sup>†</sup> For *mMBEM*, instead apply MBHAC on a random subset of  $\mathbf{X}$  to obtain parameters in Step 2.

successively merged (with a uniform probability of choosing any two clusters for merging) until all  $n$  data points have been grouped into a single cluster. Equivalently, we could also start RHAC from a specific number of clusters, and successively proceed to form larger clusters. Since we do not know the true number of clusters we run EM for all values of  $\kappa \in \mathbb{Z}^+$ , in the range  $\kappa_{\min} \leq \kappa \leq \kappa_{\max}$ . Starting with an initial choice of  $\kappa_{\max}$  number of clusters, RHAC assigns each data point randomly to any one of the clusters, with uniform assignment probability  $1/\kappa_{\max}$ . At each subsequent hierarchical agglomerative clustering stage, any two randomly picked clusters are combined, resulting in a total of  $\kappa - 1$  clusters. This process is successively repeated until all data points have been grouped into  $\kappa_{\min}$  clusters. RHAC is computationally very efficient with a fast runtime, and a low memory usage cost of  $\mathcal{O}(2n)$ .

During each trial we run the EM algorithm multiple  $(\kappa_{\max} - \kappa_{\min} + 1)$  times on the data, successively decreasing the value of  $\kappa$  by one during each run, for the entire range of  $\kappa \in \{\kappa_{\max}, \dots, \kappa_{\min}\}$ . For each  $\kappa$ , the parameters of the randomly created RHAC partitions are used to start the EM. The EM algorithm is then run iteratively, maximizing the log-likelihood estimate, until convergence to an optimal solution. The proposed multiple restart RHAC based EM (*mRHEM*) algorithm is summarized in Algorithm 1.

**Restarts using MBHAC on a random subset.** Model-based hierarchical agglomerative clustering (MBHAC) uses a Gaussian mixture model to obtain a partition of the data (Fraley, 1998; Scrucca & Raftery, 2015), and is the default EM initialization method for the `mclust` R package (Scrucca, Fop, Murphy, & Raftery, 2016). Starting with each data point of the subset in its own cluster, MBHAC merges a pair of maximum likelihood clusters at each successive stage of the hierarchical clustering, resulting in a partition for each  $\kappa \in \{n, \dots, 1\}$ . The parameters of these clusters obtained using MBHAC can then be used to initialize the EM algorithm across the desired range of  $\kappa$ .

Applying MBHAC to the full dataset is deterministic, and computationally expensive with the memory usage cost being proportional to the square of the number of data points,  $\mathcal{O}(n^2)$  (Fraley, 1998). As an alternate for large values of  $n$ , the initial model parameters can be obtained by applying MBHAC to a smaller subset of the data points chosen at random (with

uniform probability) (Fraley, 1998; Scrucca & Raftery 2015). The GMM is then fitted to all  $n$  data points by starting the EM algorithm with this choice of initial parameters.

We extend this randomized MBHAC approach to implement a multiple random restart version of the EM algorithm (*mMBEM*). Specifically, we run many trials on each dataset. For each trial we choose a random subset from among the  $n$  data points and apply MBHAC to obtain the initial EM parameters for the desired range of mixture components  $\kappa$ . Finally, we select the model with the highest BIC across all trials. The *mMBEM* algorithm is therefore identical to *mRHEM* outlined in the previous section, with the only difference being the use of MBHAC applied to a random subset to initialize the model parameters (in Step 2 of Algorithm 1).

**The Probability Estimates**

We obtain an estimate of the block connectivity probability matrix  $\hat{P}$  using the proportion of connected vertices given by our graph and using the partition  $\hat{\tau}$ . We define the  $ij$ th entry of this matrix by

$$\hat{p}_{ij} := \frac{|\{(v, w) \in E : \hat{\tau}(v) = i \text{ and } \hat{\tau}(w) = j\}|}{\hat{n}_i \hat{n}_j}, \tag{7}$$

where  $\hat{n}_i = |\{v \in V : \hat{\tau}(v) = i\}|$ . The ratio in Equation 7 defines a value from 0 to 1.

The probability estimate is compared with the original parameters that generated the graph. Recall that  $p_i$  is the proportion of vertices originally in the  $i$ th group, and  $p_{ij}$  is the probability that a specified element of the  $i$ th group has a directed edge to a specified element in the  $j$ th group. The corresponding relative error rate is defined as

$$\Delta \hat{P}_{ij} = \begin{cases} 0, & \text{for } p_{ij} = \hat{p}_{ij} = 0 \\ 2 \cdot \frac{|p_{ij} - \hat{p}_{ij}|}{p_{ij} + \hat{p}_{ij}}, & \text{otherwise.} \end{cases} \tag{8}$$

The percentage relative error in estimating the block connection probabilities is a weighted average using the class proportions,

$$\delta \hat{P} = \frac{100\%}{W} \cdot \sum_{i,j=1}^k p_i p_j \Delta \hat{P}_{ij}, \tag{9}$$

where  $W = \sum_{(i,j) \in I} p_i p_j$ , with the index set  $I = \{(i, j) : p_{ij} \neq 0, \text{ and } \hat{p}_{ij} \neq 0\}$ .

When the clustering is perfect, the expected difference  $\delta \hat{P} \approx 0.000$  because perfect clustering implies that  $\hat{p}_{ij}$  is the proportion of connected vertices in a size  $n_i n_j$  random sample from a binomial distribution with parameter  $p_{ij}$ .

**SIMULATION RESULTS**

In order to validate the effectiveness of the proposed approach, we performed multiple simulations using our surrogate connectome model. During the course of these simulations we randomly generated SBM graphs by systematically varying each of the parameters ( $n, P, \rho$ ) of our surrogate model (1), (2). For each graph we performed ASE followed by GMM-based EM clustering. We compared the effects of EM initialization on clustering performance by applying the *mRHEM* and *mMBEM* algorithms, to the same graphs, respectively. Additionally, we also tested the robustness of our model to choices of embedding dimension  $d$ , the addition of noise, and the

**Table 2.** Clustering accuracy for EM initialization using MBHAC for a single trial,  $T = 1$ . The initial parameters were obtained by applying MBHAC to all  $n$  data points.  $d$  is the number of singular values chosen for ASE. A total of 50 graphs were used for each  $n$ .

| $n$               | % Perfect clustering (% vertices misclassified) |           |           |           |           |
|-------------------|---|-----------|-----------|-----------|-----------|
|                   | $d = 2$   | $d = 3$   | $d = 4$   | $d = 5$   | $d = 6$   |
| $2^{12}$ (4,096)  | 2 (7.63)  | 0 (14.55) | 2 (15.48) | 0 (18.18) | 0 (18.23) |
| $2^{13}$ (8,192)  | 14 (1.85)                                       | 24 (7.45) | 12 (8.58) | 4 (10.83) | 2 (16.87) |
| $2^{14}$ (16,384) | 28 (1.94)                                       | 22 (2.76) | 12 (7.95) | 6 (8.11)  | 2 (7.30)  |
| $2^{15}$ (32,768) | 34 (1.65)                                       | 16 (2.76) | 12 (6.37) | 0 (5.26)  | 0 (7.52)  |

effect of varying the number of trials when applying multiple restart EM. We describe these results in detail below.

### Varying the Embedding Dimension $d$

We first assess the impact that the choice of embedding dimension has on the clustering performance when using GMM-based hierarchical clustering. We generated 50 random graphs for each value of  $n$  using the surrogate model (1), (2), and then cluster them by embedding them in  $\mathbb{R}^{2d}$  using ASE (varying the value of  $d$  each time).

For the sake of comparison, clustering was first performed by running the EM algorithm with initial parameters obtained by applying MBHAC to all  $n$  data points, implemented using the `mclust` R package (Scrucca et al., 2016). Note that applying MBHAC to all data points creates deterministic partitions resulting in just a single trial,  $T = 1$ . Table 2 shows the percentage of 50 graphs in which the vertices were perfectly clustered (i.e., each vertex  $v_i$  was correctly assigned to its true class  $\tau(v_i)$  by the algorithm) and the percentage of vertices that were misclassified across these graphs. The results indicate that using this approach to initialize the EM algorithm performed rather poorly, and was in general unsuccessful in clustering the latent vectors correctly. Interestingly, the method performed better for lower values of  $d$  and large  $n$ , with the misclassification rate being very low for these values.

Tables 3 and 4 show the results when using the proposed multiple restart variations *mMBEM*, and *mRHEM* algorithms, respectively. Both algorithms were implemented with aid of the `mclust` package. A total of 100 trials were used to cluster each graph. We observe a drastic improvement in the clustering performance when using the random multiple restart approach. Also as expected, and in contrast to MBHAC, the clustering performance improves as  $n$  increases (Athreya et al., 2016).

**Table 3.** Clustering accuracy using *mMBEM* with  $T = 100$  trials, wherein each trial was initialized using parameters obtained by applying MBHAC to a random subset of 2,000 data points. A total of 50 graphs were used for each  $n$ .

| $n$               | % Perfect clustering (% vertices misclassified) |            |            |            |            |
|-------------------|---|------------|------------|------------|------------|
|                   | $d = 2$   | $d = 3$    | $d = 4$    | $d = 5$    | $d = 6$    |
| $2^{12}$ (4,096)  | 0 (19.96)                                       | 36 (6.10)  | 14 (14.00) | 40 (0.04)  | 44 (0.04)  |
| $2^{13}$ (8,192)  | 0 (12.48)                                       | 100 (0.00) | 58 (17.36) | 98 (0.01)  | 98 (0.01)  |
| $2^{14}$ (16,384) | 14 (5.53)                                       | 100 (0.00) | 78 (18.65) | 100 (0.00) | 100 (0.00) |
| $2^{15}$ (32,768) | 100 (0.00)                                      | 100 (0.00) | 26 (20.36) | 100 (0.00) | 100 (0.00) |

**Table 4.** Clustering accuracy using *mRHEM* with  $T = 100$  trials.  $d$  is the number of singular values chosen for ASE. A total of 50 graphs were used for each  $n$ .

| $n$               | % Perfect clustering (% vertices misclassified) |            |            |            |            |
|-------------------|---|------------|------------|------------|------------|
|                   | $d = 2$   | $d = 3$    | $d = 4$    | $d = 5$    | $d = 6$    |
| $2^{12}$ (4,096)  | 50 (0.03)                                       | 46 (0.63)  | 22 (5.05)  | 10 (10.56) | 0 (9.72)   |
| $2^{13}$ (8,192)  | 100 (0.00)                                      | 100 (0.00) | 100 (0.00) | 98 (5.08)  | 92 (7.17)  |
| $2^{14}$ (16,384) | 100 (0.00)                                      | 100 (0.00) | 100 (0.00) | 100 (0.00) | 98 (3.31)  |
| $2^{15}$ (32,768) | 100 (0.00)                                      | 100 (0.00) | 100 (0.00) | 100 (0.00) | 100 (0.00) |

For the results in Table 3, the size of the random subset used for *mMBEM* initialization was kept constant at 2,000 data points, irrespective of the value of  $n$ . Rather surprisingly though, *mMBEM* performed poorly for the choice of embedding dimension  $d = 4$ , which from Figure 1 is the target dimension of interest. For the particular case of  $d = 4$ , we observed a consistent error pattern for all graphs that were not perfectly clustered. For these graphs the final clustering always resulted in  $\hat{k} = 9$ , with the largest cluster being split into two.

The clustering results improved when we increased the size of the random subset, but so did the computation time. In Table 5 we compare the performance of *mMBEM* as a function of the random subset size used for initialization, by applying it to the same 50 graphs each with  $n = 2^{15}$ , and  $d = 4$ . The average CPU elapsed time shown is the time taken to perform agglomerative hierarchical clustering given data  $X$ , and does not include the time taken to perform any other operation such as ASE, iterating EM, calculating the BICs, and so on. Doubling the size of the random subset to 4,000 data points led to approximately a sixfold increase in CPU computation time to perform randomized MBHAC, with only a marginal improvement in clustering accuracy. MBHAC initialization for subsets larger than 2,000 points results in diminishing gain.

In contrast, *mRHEM* was largely insensitive to the choice of embedding dimensionality. It was also extremely consistent in its performance with near perfect clustering accuracy for  $n \geq 2^{13}$ . While we list results for 100 trials, a larger number of *mRHEM* trials resulted in even stronger results. Furthermore, *mMBEM* is subject to an additional parameter (viz., size of the random subset used for initialization), which directly affects its clustering accuracy and computational complexity, while *mRHEM* is straightforward to implement and extremely efficient computationally. We use *mRHEM* exclusively for the remainder of the analysis.

#### Varying the Number of Vertices $n$

To examine the effects of varying  $n$  in further detail, we fixed the choice of embedding dimensionality at a constant  $d = 4$ , as selected from Figure 1. Table 6 shows the clustering performance of *mRHEM* with  $T = 100$  trials for a varying number of vertices. Misclassified vertices

**Table 5.** Average CPU<sup>†</sup> time (in seconds) taken to perform different variations of agglomerative hierarchical clustering. A total of 50 graphs were used each with  $n = 2^{15}$ , and  $d = 4$ .

| Initialization method | RHAC | MBHAC (2,000) | MBHAC (4,000) | MBHAC (8,000) | MBHAC ( $2^{15}$ )* |
|-----------------------|------|---------------|---------------|---------------|---------------------|
| CPU time (secs.)      | 7.36 | 1.87          | 12.19         | 83.22         | 4,554.31            |
| % Perfect clustering  | 100  | 26            | 38            | 72            | 12*                 |

<sup>†</sup> Desktop AMD Ryzen 2700x (3.7 GHz) with 32 GB RAM (DDR4, 3200 MHz), and *mclust* version 5.4.2.

\* Applying MBHAC to all  $n$  points, results in a single trial.

**Table 6.** Varying  $n$ : Clustering accuracy using  $mRHEM$  with  $T = 100$  trials for  $d = 4$ , as the number of vertices  $n$  is increased while keeping other parameters constant. A total of 50 graphs were used for each  $n$ .

| $n$               | $\% \hat{k} = 8$ | Perfect classification % | When imperfect classification          |                           | Overall Avg. ARI |
|-------------------|------------------|--------------------------|--|---------------------------|------------------|
|                   |                  |                          | Avg. number (%) misclassified vertices | Avg. $\delta \hat{P}(\%)$ |                  |
| $2^{11}$ (2,048)  | 14               | 0                        | 315.60 (15.41%)                        | 47.385                    | 0.9032           |
| $2^{12}$ (4,096)  | 56               | 22                       | 206.95 (5.05%)                         | 1.510                     | 0.9346           |
| $2^{13}$ (8,192)  | 100              | 100                      | 0                                      | 0.000                     | 1.0000           |
| $2^{14}$ (16,384) | 100              | 100                      | 0                                      | 0.000                     | 1.0000           |
| $2^{15}$ (32,768) | 100              | 100                      | 0                                      | 0.000                     | 1.0000           |

were measured from maximal BIC among trials, and averaged over 50 graphs. Additionally, we also include the percentage relative error in estimating the block connection probabilities (9), and measure the adjusted Rand index (ARI) (Hubert & Arabie, 1985). Here the ARI was calculated in comparison to the true class memberships, and serves as an estimate for the overall accuracy of classification. ARI is a popular similarity score for comparing two partitioning schemes for the same data points, with a higher value of ARI indicating high similarity; 1 indicating that they are identical; and 0 for randomly generated partitions.

**Varying the Proportions  $\rho$**

To test the robustness of the approach, we varied the SBM parameters, such that first  $\rho = (\rho_1, \dots, \rho_k)$  was varied while keeping  $P$  constant, and then  $P$  was varied while keeping  $\rho$  constant. To vary the class proportions we used a Dirichlet distribution  $Dir(r_\rho \cdot \rho + J_{1,k})$ , where  $r_\rho$  is a constant, and  $J_{i,j}$  is an  $i \times j$  matrix of all ones. When  $r_\rho = \infty$  we have the original membership proportions in (2), and when  $r_\rho = 0$  the proportions are sampled from a uniform distribution. Table 7 shows the clustering results using  $mRHEM$  with 100 trials as  $\rho$  was varied. A total of 50 graphs were generated for each  $\rho$ , while keeping  $P$ ,  $n = 2^{14}$ , and  $d = 4$  constant for each graph. We include the data for  $r = \infty$  for comparison.

**Varying the Probability Matrix  $P$**

To vary the connectivity probability matrix we used another Dirichlet distribution centered on  $P$ , with parameter  $r_p$ , such that the probabilities are sampled from a uniform distribution when

**Table 7.** Varying  $\rho$ : Clustering accuracy using  $mRHEM$  with 50 graphs and  $T = 100$  trials, with varied block membership proportions. Total number of vertices was kept constant  $n = 2^{14}$ , and  $d = 4$ .

| $r_\rho$ | $\% \hat{k} = 8$ | Perfect classification % | When imperfect classification          |                           | Overall Avg. ARI |
|----------|------------------|--------------------------|--|---------------------------|------------------|
|          |                  |                          | Avg. number (%) misclassified vertices | Avg. $\delta \hat{P}(\%)$ |                  |
| $\infty$ | 100              | 100                      | 0                                      | 0.000                     | 1.0000           |
| 10,000   | 100              | 100                      | 0                                      | 0.000                     | 1.0000           |
| 1,000    | 100              | 100                      | 0                                      | 0.000                     | 1.0000           |
| 100      | 100              | 100                      | 0                                      | 0.000                     | 1.0000           |
| 10       | 100              | 100                      | 0                                      | 0.000                     | 1.0000           |
| 0        | 78               | 78                       | 3,026.455 (18.47%)                     | 2.743                     | 0.9960           |

$r_p = 0$ , and is given by the matrix  $P$  when  $r_p = \infty$ . Additionally, to ensure that the sampled graphs remain sparse we put bounds on the Dirichlet sampled  $ij$ th entry of the probability matrix,  $p_{ij}^D$ , such that

$$\max(0, p_{ij} - 0.2) \leq p_{ij}^D \leq p_{ij} + 0.2. \tag{10}$$

Table 8 shows the clustering results for varying  $P$  while keeping  $\rho$  constant for  $n = 4,096$ . Alternatively, when the number of vertices is increased to  $n = 8,192$ , we observed that the  $mRHEM$  performance did not essentially deteriorate as block connection probabilities were varied relative to the original values; when the number of vertices is set to  $n = 16,384$ ,  $mRHEM$  achieves perfect classification over the entire range of  $r_p$ .

**Effect of Adding Noise**

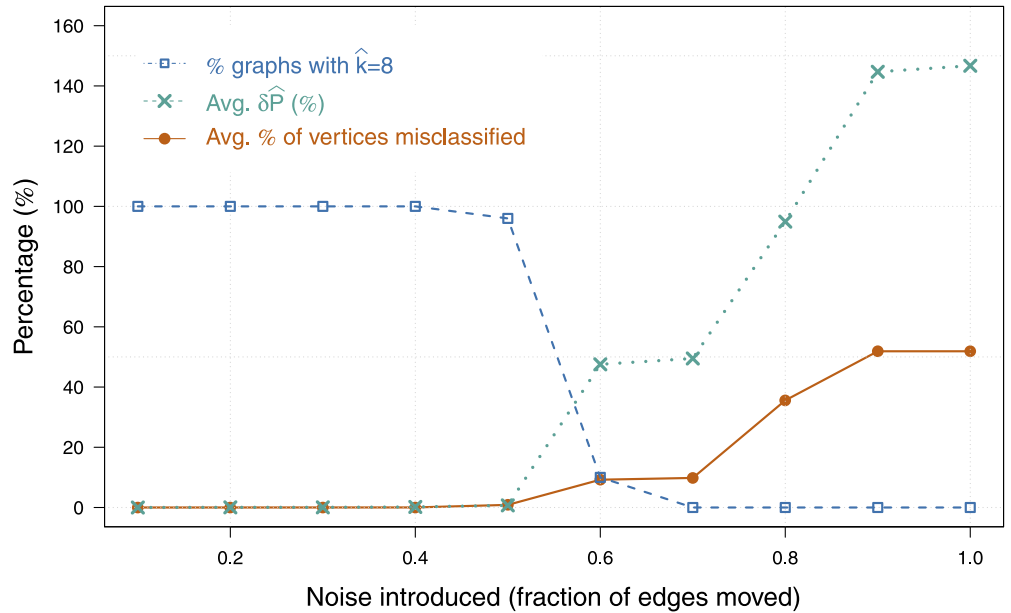
To test the tolerance of the proposed clustering algorithm under experimentally realistic model misspecification, we simulate errors in pre- or postsynaptic neuron identification. In order to do this we add noise to our model by randomly moving edges within the adjacency matrix. Specifically, a directed edge in the adjacency matrix is moved by flipping the corresponding 1 into a 0, and simultaneously flipping a randomly chosen 0 somewhere else in the matrix into a 1. Therefore, the total number of edges before and after the addition of noise in a graph remains the same.

The percentage of edge misspecification in a noisy graph indicates the fraction of edges, relative to the total number of edges in the graph, that are moved. The percentage misspecification thus determines the size of the subset of edges moved. The subset of edges (and corresponding subset of non-edges) to be flipped are chosen using a uniform random distribution among all possible subsets of the determined size. This ensures that over several instances of random noisy graphs, the average number of edges removed from each pair of neuronal classes is proportional to the total number of connections (edges) in between that pair of classes. Since the graph is sparse, the average number of corresponding edges added has comparatively small differences across different pairs of neuronal classes. Consequentially, on average, pairs of neuronal classes with more connections have more noise introduced.

We measured how well  $mRHEM$  with  $T = 100$  trials was able to estimate the original class assignment in the presence of noise. Figure 3 shows the average classification accuracy as a function of the fraction of edges moved, for a total of 10 graphs each with  $n = 2^{14}$ , and  $d = 4$ . The clustering results demonstrate  $mRHEM$  to be extremely tolerant towards added noise, with near perfect classification even with 50% edge misspecification.

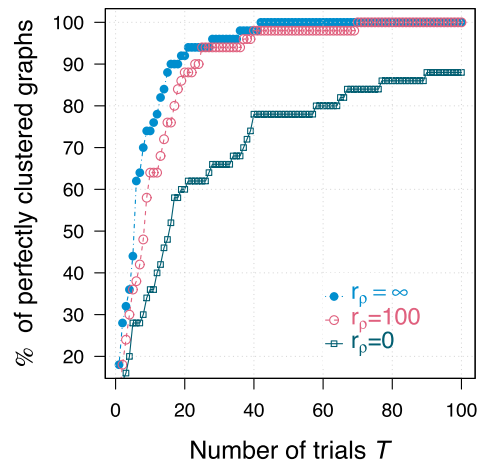
**Table 8.** Varying  $P$ : Clustering accuracy for using  $mRHEM$  with 50 graphs and  $T = 100$  trials, with varied block connection probabilities. Total number of vertices was kept constant  $n = 2^{12}$ , and  $d = 4$ .

| $r_p$    | % $\hat{k} = 8$ | Perfect classification % | When imperfect classification          |                          | Overall Avg. ARI |
|----------|-----------------|--------------------------|--|--------------------------|------------------|
|          |                 |                          | Avg. number (%) misclassified vertices | Avg. $\delta\hat{P}$ (%) |                  |
| $\infty$ | 56              | 22                       | 206.95 (5.05%)                         | 1.510                    | 0.9346           |
| 10,000   | 44              | 20                       | 252.35 (6.16%)                         | 11.184                   | 0.9116           |
| 1,000    | 60              | 14                       | 162.05 (3.96%)                         | 11.000                   | 0.9636           |
| 100      | 84              | 32                       | 62.71 (1.53%)                          | 6.754                    | 0.9954           |
| 10       | 100             | 100                      | 0                                      | 0.000                    | 1.0000           |
| 0        | 100             | 100                      | 0                                      | 0.000                    | 1.0000           |



**Figure 3.** Adding noise: Average percentage of vertices that were misclassified versus the amount of noise introduced, that is, fraction of edges moved. Also shown are the percentage of graphs whose clustering resulted in correctly estimating  $\hat{k} = 8$ , and relative error  $\delta\hat{P}$ , averaged over all graphs.

The model’s robustness to noise is partly attributed to the fact that not all neuron types contribute equally to the network connectivity. If  $\rho$  is skewed with disproportionately sized groups, then the process of flipping random edges has a higher probability (than evenly sized classes) that the removal and addition happens within the same pair of vertex classes. Similarly, the greater the differences among entries in  $P$ , the more robust the clustering is to the addition of noise. More generally, asymmetry in the parameter specifications increases the tolerance of the model to edge misspecification.

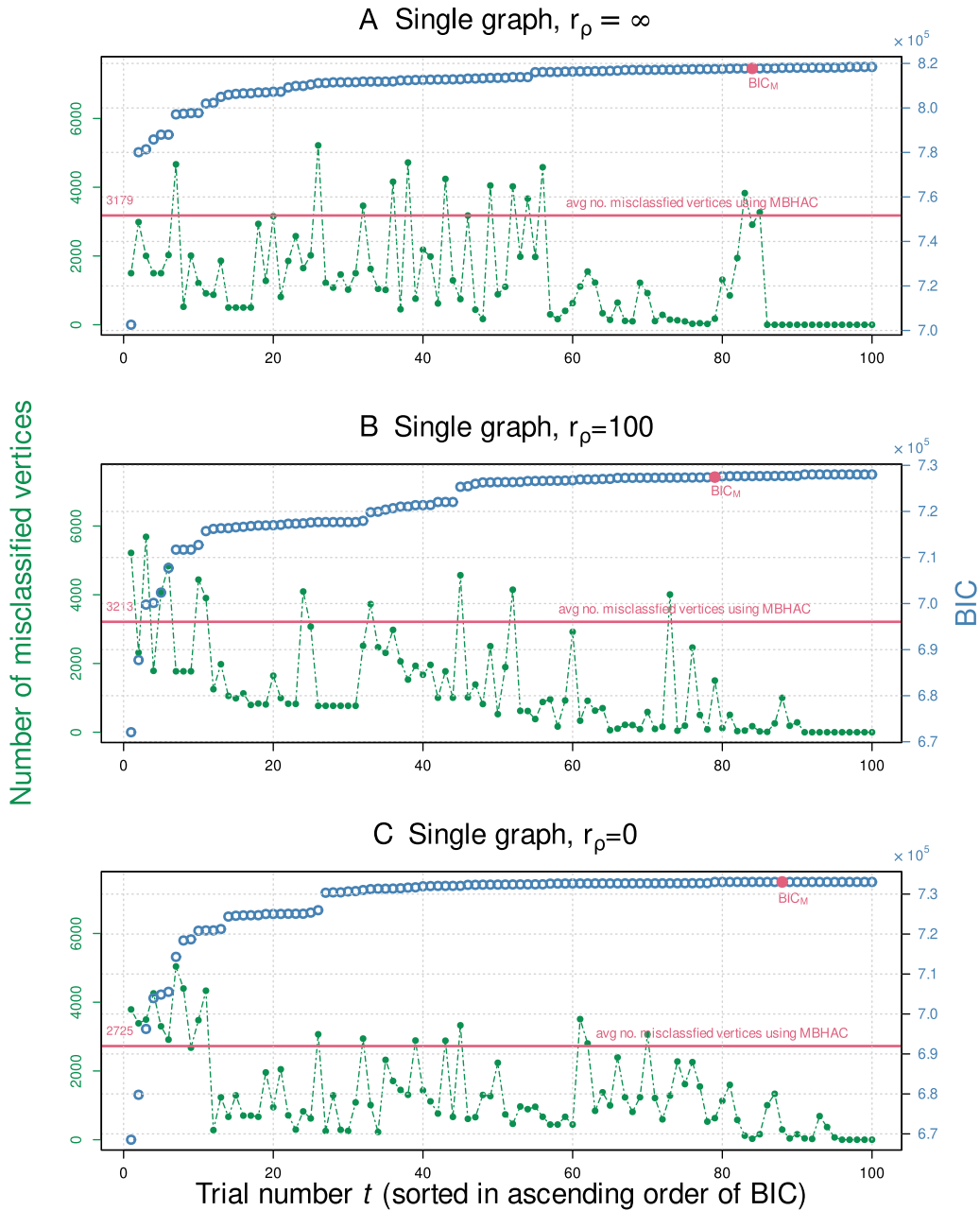


**Figure 4.** Varying number of trials: Percentage of perfectly clustered graphs when running  $T$  trials of  $m$ RHEM. A total of 50 graphs were used for different values of  $r_p$  (with  $r_p = \infty$ ,  $n = 2^{14}$ , and  $d = 4$  held constant).



**Influence of Number of Trials on mRHEM Performance**

A fundamental disadvantage of using multiple restart EM is the computational cost associated with running multiple trials. To the best of our knowledge there is no theoretical solution available in the literature to determine the number of random initializations that would be sufficient to ensure a full examination of the likelihood function (Biernacki et al., 2003; Shireman et al., 2017). In the absence of an analytical solution, we perform an empirical analysis to help determine the number of trials needed for mRHEM to converge to an optimal solution. Figure 4



**Figure 5.** Number of misclassified vertices versus mRHEM trial number for a single graph for (A)  $r_\rho = \infty$ , (B)  $r_\rho = 100$ , and (C)  $r_\rho = 0$  (with  $r_p = \infty$ ,  $n = 2^{14}$ , and  $d = 4$  held constant). The trials are sorted in increasing magnitude of  $BIC^{(t)}$ . Also, shown for comparison is  $BIC_M$  corresponding to initialization using MBHAC applied to all  $n$  data points.

shows the percentage of graphs that are perfectly clustered as a function of the number of trials used to run *mRHEM*. Only 37 trials were needed to achieve perfect clustering for over 95% of the graphs for  $r_p = \infty$ , and  $r_p = 100$ .

We also investigate the empirical relation between BIC and clustering accuracy, as a function of the number of trials. For a single randomly chosen graph generated with the original parameters, Figure 5A shows the number of misclassified vertices and the resulting BIC values for 100 trials of *mRHEM*. The trials have been sorted along the horizontal axis in ascending order of their resulting  $BIC^{(t)}$  values, such that the random trial with the lowest BIC corresponds to  $t = 1$ , while the random trial with the highest BIC is  $t = 100$ . Also, shown on the same plot is the misclassification error and corresponding  $BIC_M$  value when initializing using MBHAC on the same graph. A similar comparison is done for a single graph generated with  $r_p = 100$  (Figure 5B) and for a single graph generated with  $r_p = 0$  (Figure 5C).

We observe from Figure 5 that while  $BIC_M > BIC^{(t)}$  for  $\approx 80\%$  of the trials, its ability to successfully predict class assignment is worse than  $\approx 90\%$  of the *mRHEM* trials, evidenced by the small number of data points among the *mRHEM* trials above the horizontal (pink) line indicating the number of misclassified vertices when initializing using MBHAC.  $BIC_M$  could be used as a reference when deciding whether additional trials are needed. If the BIC values of all random trials are less than  $BIC_M$ , more trials may be needed. Finally, we choose the model with the highest BIC. Note that the number of misclassifications is not a monotonic function w.r.t. BIC, that is, a higher BIC does not necessarily guarantee better clustering.

The time penalty and availability of computational resources are other important factors to consider when choosing the number of random trials. Despite the added computational cost associated with running EM several times, 100 *mRHEM* trials entails only a contained ( $\approx 270\%$  on average) increase in CPU computation time. Additionally, since *mRHEM* is performing multiple quick trials, it allows for a relatively easy parallel-processing implementation (as opposed to one intensive trial using MBHAC). This could allow future CPU-intensive calculations to be performed simultaneously, resulting in significant time savings for *mRHEM*.

## DISCUSSION

Understanding the types of neurons that comprise nervous systems is a fundamental step towards a more comprehensive understanding of neural circuits (Armañanzas & Ascoli, 2015). The need for cell type classification from brain data is demonstrated by it being the first high-priority research area identified by the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative working group interim report (NIH, 2013) and the resulting launch of the BRAIN Initiative Cell Census Network (<https://biccn.org>). Previous approaches to classifying cell types have largely focused on the analysis of morphological, physiological, or genetic properties. Here, we promote a complementary strategy that directly leverages connectivity. Our methodology effectively recovered the true number of clusters and cluster assignments as the number of vertices increased, even under experimentally realistic model misspecifications, corroborating its potential utility for analyzing real connectomic data.

Neuronal classification has traditionally relied on axonal and dendritic morphology, molecular expression, and electrophysiology for characterizing cellular properties in the nervous system (Petilla Interneuron Nomenclature Group et al., 2008). On the one hand, the expedient abundance of such data has allowed the creation of increasingly unbiased descriptive taxonomies (DeFelipe et al., 2013; Yuste et al., 2020). On the other, these experimentally accessible dimensions are only indirect proxies for the mechanistically more relevant features of network

connectivity, developmental control, and experience-dependent plasticity (Armañanzas & Ascoli, 2015; Shepherd et al., 2019). In particular, a community consensus has been coalescing that the complete synaptic circuitry of a neural system constitutes the fundamental architectural underpinning of its in vivo dynamics and functions (Abbott et al., 2020). From this perspective, a quantitative specification of neuron types based on network connectivity such as that proposed in this work may constitute the most fundamental parts list for deconstructing brain computation. This raises the important question of mapping the connectomics-based neuron classification to other well-studied biological dimensions, including transcriptomics and spiking activity patterns. Addressing this problem remains an open challenge in neuroscience.

Our ability as a community to estimate connectomes from real brain data has recently been transformed by breathtaking advances in techniques such as nanoscale electron microscopy (Bock et al., 2011; Denk & Horstmann, 2004; Jarrell et al., 2012; Takemura et al., 2013), structural multicolor microscale light microscopy (Livet et al., 2007) paired with tissue clearing (Chung & Deisseroth, 2013), functional mesoscale light microscopy (Ahrens, Orger, Robson, Li, & Keller, 2013; Schrödel, Prevedel, Aumayr, Zimmer, & Vaziri, 2013), macroscale functional and diffusion magnetic resonance imaging (Craddock et al., 2013), computational morphology and anatomy (Peng et al., 2017; Ropireddy & Ascoli, 2011), and optical coherence tomography (Magnain et al., 2014). These technological breakthroughs require new approaches to analyze the resulting data, at scale, using principled statistical tools.

Our work illustrates the value of graph theoretic tools for discovering and assigning cell types in large scale simulations using connectivity information alone (Ascoli & Atkeson, 2005). In particular, we show that these methods can be used to recover class assignment for neural cells connected in biologically plausible proportions, at practical graph sizes for which data are emerging. The analysis and results of these surrogate data suggest that, at least in some circumstances, applying singular value decomposition and clustering techniques to the adjacency matrix rather than to its Laplacian results in consistent outcomes. However, there is a clear need for a theoretical framework that guarantees convergence for data that are asymmetric adjacency matrices representing directed graphs.

For GMM-based EM clustering of the surrogate data, the proposed *mRHEM* approach heavily outperforms the default MBHAC initialization used by `mc1ust` (Scrucca et al., 2016; Scrucca & Raftery, 2015). We show that initializing the EM algorithm with random hierarchical agglomerative clustering multiple times is more effective than standard model-based hierarchical clustering at identifying the correct classification, as quantified by key measures of accuracy, such as clustering into the correct number of groups and misclassifying as few vertices as possible.

While the proposed approach scales extremely well for large networks with  $2^{12} \leq n \leq 2^{15}$  vertices, a practical limitation of applying our SBM inference model to real connectomic data is that it requires the size of the dataset (number of vertices in the network) to be much larger than the number of model parameters. The number of model parameters grows linearly with the number of blocks  $k$  and depends quadratically on the embedding dimension  $d$  (5). While performing ASE on a sparse graph ensures  $d \ll n$ , there is no guarantee that  $k \ll n$  holds for real data. Our described approach would still attempt to find the most parsimonious model (smallest  $k$ ) that fits the given data. Recent attempts of applying the SBM framework with small values of  $k$  to model connectomic data (Priebe et al., 2017, 2019) and detect community structure (Betzel et al., 2018; Faskowitz et al., 2018; Moyer et al., 2015; Pavlovic et al., 2014) have yielded promising results. It remains necessary, however, to further examine the relationship between the parameter  $k$  and the required  $n$  to accurately model networks with even wider range and complexity. Such assessments could drive experimental efforts to reach benchmarked data-collection goals.

In future work, we will extend these results both theoretically and methodologically. We hope to characterize the circumstances in which one could expect better performance by *m*RHEM compared with MBHAC, and in particular find a probabilistic characterization of the optimal number of trials needed to obtain perfect clustering. We also intend to extend these results to include not only connectivity information, but also various other vertex and edge attributes of the network, such as spatial, morphological, electrophysiological, and genetic properties. While the contribution of this paper was methodological in scope, the lack of experimental validation at this time prevents a definitive assessment of its full scientific impact. Future work will strive to apply the approach introduced here to estimated connectomes from biological data, allowing an empirical test of its ability to foster novel neuroscientific insights.

### SUPPORTING INFORMATION

**R code:** Self-contained R-script [*.r* filetype] to generate surrogate data and replicate all simulation results described in the article is available at [https://doi.org/10.1162/netn\\_a\\_00195](https://doi.org/10.1162/netn_a_00195).

### AUTHOR CONTRIBUTIONS

Ketan Mehta: Conceptualization; Investigation; Methodology; Software; Validation; Visualization; Writing – original draft; Writing – review & editing. Rebecca F. Goldin: Conceptualization; Funding acquisition; Investigation; Methodology; Software; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing. David Marchette: Conceptualization; Investigation; Methodology; Software; Validation; Visualization; Writing – original draft; Writing – review & editing. Joshua T. Vogelstein: Conceptualization; Investigation; Methodology; Validation; Visualization; Writing – original draft; Writing – review & editing. Carey E. Priebe: Conceptualization; Investigation; Methodology; Validation; Visualization; Writing – original draft; Writing – review & editing. Giorgio A. Ascoli: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

### FUNDING INFORMATION

Giorgio A. Ascoli, National Institutes of Health (<https://dx.doi.org/10.13039/100000002>), Award ID: R01NS39600. Giorgio A. Ascoli, National Institutes of Health (<https://dx.doi.org/10.13039/100000002>), Award ID: U01MH114829. Rebecca F. Goldin, National Science Foundation (<https://dx.doi.org/10.13039/100000001>), Award ID: 1201458.

### REFERENCES

- Abbe, E. (2017). Community detection and stochastic block models: Recent developments. *Journal of Machine Learning Research*, 18(1), 6446–6531. <https://dl.acm.org/doi/10.5555/3122009.3242034>
- Abbott, L. F., Bock, D. D., Callaway, E. M., Denk, W., Dulac, C., Fairhall, A. L., ... Van Essen, D. C. (2020). The mind of a mouse. *Cell*, 182(6), 1372–1376. <https://doi.org/10.1016/j.cell.2020.08.010>, PubMed: 32946777
- Ahrens, M. B., Orger, M. B., Robson, D. N., Li, J. M., & Keller, P. J. (2013). Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nature Methods*, 10(5), 413–420. <https://doi.org/10.1038/nmeth.2434>, PubMed: 23524393
- Armañanzas, R., & Ascoli, G. A. (2015). Towards the automatic classification of neurons. *Trends in Neurosciences*, 38(5), 307–318. <https://doi.org/10.1016/j.tins.2015.02.004>, PubMed: 25765323
- Ascoli, G. A., & Atkeson, J. C. (2005). Incorporating anatomically realistic cellular-level connectivity in neural network models of the rat hippocampus. *Biosystems*, 79(1), 173–181. <https://doi.org/10.1016/j.biosystems.2004.09.024>, PubMed: 15649602
- Athreya, A., Priebe, C. E., Tang, M., Lyzinski, V., Marchette, D. J., & Sussman, D. L. (2016). A limit theorem for scaled eigenvectors of random dot product graphs. *Sankhya A*, 78(1), 1–18. <https://doi.org/10.1007/s13171-015-0071-x>

- Attili, S. M., Mackesey, S. T., & Ascoli, G. A. (2020). Operations research methods for estimating the population size of neuron types. *Annals of Operations Research*, 289, 33–50. <https://doi.org/10.1007/s10479-020-03542-7>, PubMed: 33343053
- Betzal, R. F., Medaglia, J. D., & Bassett, D. S. (2018). Diversity of meso-scale architecture in human and non-human connectomes. *Nature Communications*, 9(346), 1–14. <https://doi.org/10.1038/s41467-017-02681-z>, PubMed: 29367627
- Biernacki, C., Celeux, G., & Govaert, G. (2003). Choosing starting values for the EM algorithm for getting the highest likelihood in multivariate Gaussian mixture models. *Computational Statistics and Data Analysis*, 41(3), 561–575. [https://doi.org/10.1016/S0167-9473\(02\)00163-9](https://doi.org/10.1016/S0167-9473(02)00163-9)
- Bock, D. D., Lee, W.-C. A., Kerlin, A. M., Andermann, M. L., Hood, G., Wetzel, A. W., ... Reid, R. C. (2011). Network anatomy and in vivo physiology of visual cortical neurons. *Nature*, 471(7337), 177–182. <https://doi.org/10.1038/nature09802>, PubMed: 21390124
- Christopoulos, D. (2016). Introducing unit invariant knee (UIK) as an objective choice for elbow point in multivariate data analysis techniques. Available at SSRN 3043076. <https://doi.org/10.2139/ssrn.3043076>
- Chung, K., & Deisseroth, K. (2013). CLARITY for mapping the nervous system. *Nature Methods*, 10(6), 508–513. <https://doi.org/10.1038/nmeth.2481>, PubMed: 23722210
- Craddock, R. C., Jbabdi, S., Yan, C.-G., Vogelstein, J. T., Castellanos, F. X., Di Martino, A., ... Milham, M. P. (2013). Imaging human connectomes at the macroscale. *Nature Methods*, 10(6), 524–539. <https://doi.org/10.1038/nmeth.2482>, PubMed: 23722212
- DeFelipe, J., López-Cruz, P. L., Benavides-Piccione, R., Bielza, C., Larrañaga, P., Anderson, S., ... Ascoli, G. A. (2013). New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nature Reviews Neuroscience*, 14(3), 202–216. <https://doi.org/10.1038/nrn3444>, PubMed: 23385869
- Denk, W., & Horstmann, H. (2004). Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biology*, 2(11), e329. <https://doi.org/10.1371/journal.pbio.0020329>, PubMed: 15514700
- Faskowitz, J., Yan, X., Zuo, X.-N., & Sporns, O. (2018). Weighted stochastic block models of the human connectome across the life span. *Scientific Reports*, 8(1), 1–16. <https://doi.org/10.1038/s41598-018-31202-1>, PubMed: 30158553
- Fishkind, D. E., Sussman, D. L., Tang, M., Vogelstein, J. T., & Priebe, C. E. (2013). Consistent adjacency-spectral partitioning for the stochastic block model when the model parameters are unknown. *SIAM Journal on Matrix Analysis and Applications*, 34(1), 23–39. <https://doi.org/10.1137/120875600>
- Fraley, C. (1998). Algorithms for model-based Gaussian hierarchical clustering. *SIAM Journal on Scientific Computing*, 20(1), 270–281. <https://doi.org/10.1137/S1064827596311451>
- Funke, T., & Becker, T. (2019). Stochastic block models: A comparison of variants and inference methods. *PLoS ONE*, 14(4), e0215296. <https://doi.org/10.1371/journal.pone.0215296>, PubMed: 31013290
- Hamilton, D., Shepherd, G., Martone, M., & Ascoli, G. (2012). An ontological approach to describing neurons and their relationships. *Frontiers in Neuroinformatics*, 6, 1–15. <https://doi.org/10.3389/fninf.2012.00015>, PubMed: 22557965
- Holland, P. W., Laskey, K. B., & Leinhardt, S. (1983). Stochastic blockmodels: First steps. *Social Networks*, 5(2), 109–137. [https://doi.org/10.1016/0378-8733\(83\)90021-7](https://doi.org/10.1016/0378-8733(83)90021-7)
- Holland, P. W., & Leinhardt, S. (1981). An exponential family of probability distributions for directed graphs. *Journal of the American Statistical Association*, 76(373), 33–50. <https://doi.org/10.1080/01621459.1981.10477598>
- Hubert, L., & Arabie, P. (1985). Comparing partitions. *Journal of Classification*, 2(1), 193–218. <https://doi.org/10.1007/BF01908075>
- Jarrell, T. A., Wang, Y., Bloniarz, A. E., Brittin, C. A., Xu, M., Thomson, J. N., ... Emmons, S. W. (2012). The connectome of a decision-making neural network. *Science*, 337(6093), 437–444. <https://doi.org/10.1126/science.1221762>, PubMed: 22837521
- Kwedlo, W. (2015). A new random approach for initialization of the multiple restart EM algorithm for Gaussian model-based clustering. *Pattern Analysis and Applications*, 18(4), 757–770. <https://doi.org/10.1007/s10044-014-0441-3>
- Livet, J., Weissman, T. A., Kang, H., Draft, R. W., Lu, J., Bennis, R. A., ... Lichtman, J. W. (2007). Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature*, 450(7166), 56–62. <https://doi.org/10.1038/nature06293>, PubMed: 17972876
- Magnain, C., Augustinack, J. C., Reuter, M., Wachinger, C., Frosch, M. P., Ragan, T., ... Fischl, B. (2014). Blockface histology with optical coherence tomography: A comparison with Nissl staining. *NeuroImage*, 84, 524–533. <https://doi.org/10.1016/j.neuroimage.2013.08.072>, PubMed: 24041872
- Marchette, D., Priebe, C., & Coppersmith, G. (2011). Vertex nomination via attributed random dot product graphs. *Proceedings of the 57th ISI World Statistics Congress*, 6, 16.
- McDaid, A. F., Murphy, T. B., Friel, N., & Hurley, N. J. (2013). Improved Bayesian inference for the stochastic block model with application to large networks. *Computational Statistics and Data Analysis*, 60, 12–31. <https://doi.org/10.1016/j.csda.2012.10.021>
- Melnykov, V., & Melnykov, I. (2012). Initializing the EM algorithm in Gaussian mixture models with an unknown number of components. *Computational Statistics and Data Analysis*, 56(6), 1381–1395. <https://doi.org/10.1016/j.csda.2011.11.002>
- Moyer, D., Gutman, B., Prasad, G., Faskowitz, J., Steeg, G. V., & Thompson, P. (2015). Blockmodels for connectome analysis. In *11th international symposium on medical information processing and analysis* (Vol. 9681, pp. 62–70). Cuenca, Ecuador: SPIE. <https://doi.org/10.1117/12.2211519>
- NIH. (2013). *Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative working group interim report*. Retrieved from [https://braininitiative.nih.gov/sites/default/files/pdfs/11252013\\_interim\\_report\\_exec\\_sum\\_508c.pdf](https://braininitiative.nih.gov/sites/default/files/pdfs/11252013_interim_report_exec_sum_508c.pdf)
- Pavlovic, D. M., Vértes, P. E., Bullmore, E. T., Schafer, W. R., & Nichols, T. E. (2014). Stochastic blockmodeling of the modules and core of the *Caenorhabditis elegans* connectome. *PLoS ONE*, 9(7), e97584. <https://doi.org/10.1371/journal.pone.0097584>, PubMed: 24988196
- Peng, H., Zhou, Z., Meijering, E., et al. (2017). Automatic tracing of ultra-volumes of neuronal images. *Nature Methods*, 14, 332–333. <https://doi.org/10.1038/nmeth.4233>
- Petilla Interneuron Nomenclature Group, Ascoli, G., Alonson-Nanclares, L., Anderson, S. A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., ... Yuste, R. (2008). Petilla terminology:

- Nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nature Reviews Neuroscience*, 9(7), 557–568. <https://doi.org/10.1038/nrn2402>, PubMed: 18568015
- Priebe, C. E., Park, Y., Tang, M., Athreya, A., Lyzinski, V., Vogelstein, J. T., ... Cardona, A. (2017). Semiparametric spectral modeling of the *Drosophila* connectome. *arXiv:1705.03297*. <https://arxiv.org/abs/1705.03297>
- Priebe, C. E., Park, Y., Vogelstein, J. T., Conroy, J. M., Lyzinski, V., Tang, M., ... Bridgeford, E. (2019). On a two-truths phenomenon in spectral graph clustering. *Proceedings of the National Academy of Sciences*, 116(13), 5995–6000. <https://doi.org/10.1073/pnas.1814462116>, PubMed: 30850525
- Rohe, K., Chatterjee, S., & Yu, B. (2011). Spectral clustering and the high-dimensional stochastic blockmodel. *The Annals of Statistics*, 39(4), 1878–1915. <https://doi.org/10.1214/11-AOS887>
- Ropireddy, D., & Ascoli, G. (2011). Potential synaptic connectivity of different neurons onto pyramidal cells in a 3d reconstruction of the rat hippocampus. *Frontiers in Neuroinformatics*, 5, 1–5. <https://doi.org/10.3389/fninf.2011.00005>, PubMed: 21779242
- Satopaa, V., Albrecht, J., Irwin, D., & Raghavan, B. (2011). Finding a “Kneedle” in a haystack: Detecting knee points in system behavior. In *31st International Conference on Distributed Computing Systems Workshops* (pp. 166–171). Minneapolis, MN, USA. <https://doi.org/10.1109/ICDCSW.2011.20>
- Scheinerman, E. R., & Tucker, K. (2010). Modeling graphs using dot product representations. *Computational Statistics*, 25(1), 1–16. <https://doi.org/10.1007/s00180-009-0158-8>
- Schrödel, T., Prevedel, R., Aumayr, K., Zimmer, M., & Vaziri, A. (2013). Brain-wide 3D imaging of neuronal activity in *Caenorhabditis elegans* with sculpted light. *Nature Methods*, 10(10), 1013–1020. <https://doi.org/10.1038/nmeth.2637>, PubMed: 24013820
- Scrucca, L., Fop, M., Murphy, T. B., & Raftery, A. E. (2016). Mclust 5: Clustering, classification and density estimation using Gaussian finite mixture models. *The R Journal*, 8(1), 289–317. <https://doi.org/10.32614/RJ-2016-021>, PubMed: 27818791
- Scrucca, L., & Raftery, A. E. (2015). Improved initialisation of model-based clustering using Gaussian hierarchical partitions. *Advances in Data Analysis and Classification*, 9(4), 447–460. <https://doi.org/10.1007/s11634-015-0220-z>, PubMed: 26949421
- Shepherd, G. M., Marenco, L., Hines, M. L., Migliore, M., McDougal, R. A., Carnevale, N. T., ... Ascoli, G. A. (2019). Neuron names: A gene- and property-based name format, with special reference to cortical neurons. *Frontiers in Neuroanatomy*, 13, 1–25. <https://doi.org/10.3389/fnana.2019.00025>, PubMed: 30949034
- Shireman, E., Steinley, D., & Brusco, M. J. (2017). Examining the effect of initialization strategies on the performance of Gaussian mixture modeling. *Behavior Research Methods*, 49(1), 282–293. <https://doi.org/10.3758/s13428-015-0697-6>, PubMed: 26721666
- Sussman, D. L., Tang, M., Fishkind, D. E., & Priebe, C. E. (2012). A consistent adjacency spectral embedding for stochastic blockmodel graphs. *Journal of the American Statistical Association*, 107(499), 1119–1128. <https://doi.org/10.1080/01621459.2012.699795>
- Takemura, S. Y., Bharioke, A., Lu, Z., Nern, A., Vitaladevuni, S., Rivlin, P. K., ... Chklovskii, D. B. (2013). A visual motion detection circuit suggested by *Drosophila* connectomics. *Nature*, 500(7461), 175–181. <https://doi.org/10.1038/nature12450>, PubMed: 23925240
- Tecuatl, C., Wheeler, D. W., Sutton, N., & Ascoli, G. A. (2020). Comprehensive estimates of potential synaptic connections in local circuits of the rodent hippocampal formation by axonal-dendritic overlap. *Journal of Neuroscience*, 41(8), 1665–1683. <https://doi.org/10.1523/JNEUROSCI.1193-20.2020>, PubMed: 33361464
- Vogelstein, J. T., Bridgeford, E. W., Pedigo, B. D., Chung, J., Levin, K., Mensh, B., & Priebe, C. E. (2019). Connectal coding: Discovering the structures linking cognitive phenotypes to individual histories. *Current Opinion in Neurobiology*, 55, 199–212. <https://doi.org/10.1016/j.conb.2019.04.005>, PubMed: 31102987
- Wheeler, D. W., White, C. M., Rees, C. L., Komendantov, A. O., Hamilton, D. J., & Ascoli, G. A. (2015). Hippocampome.org: A knowledge base of neuron types in the rodent hippocampus. *eLife*, 4, e09960. <https://doi.org/10.7554/eLife.09960>, PubMed: 26402459
- Yuste, R., Hawrylycz, M., Aalling, N., Aguilar-Valles, A., Arendt, D., Armañanzas, R., ... Lein, E. (2020). A community-based transcriptomics classification and nomenclature of neocortical cell types. *Nature Neuroscience*, 23(12), 1456–1468. <https://doi.org/10.1038/s41593-020-0685-8>, PubMed: 32839617