

# Benchmarking and Refining Cell-Cell Interactions with Spatial Transcriptomics and Deep Learning



Mihir Bafna  
Georgia Institute of Technology

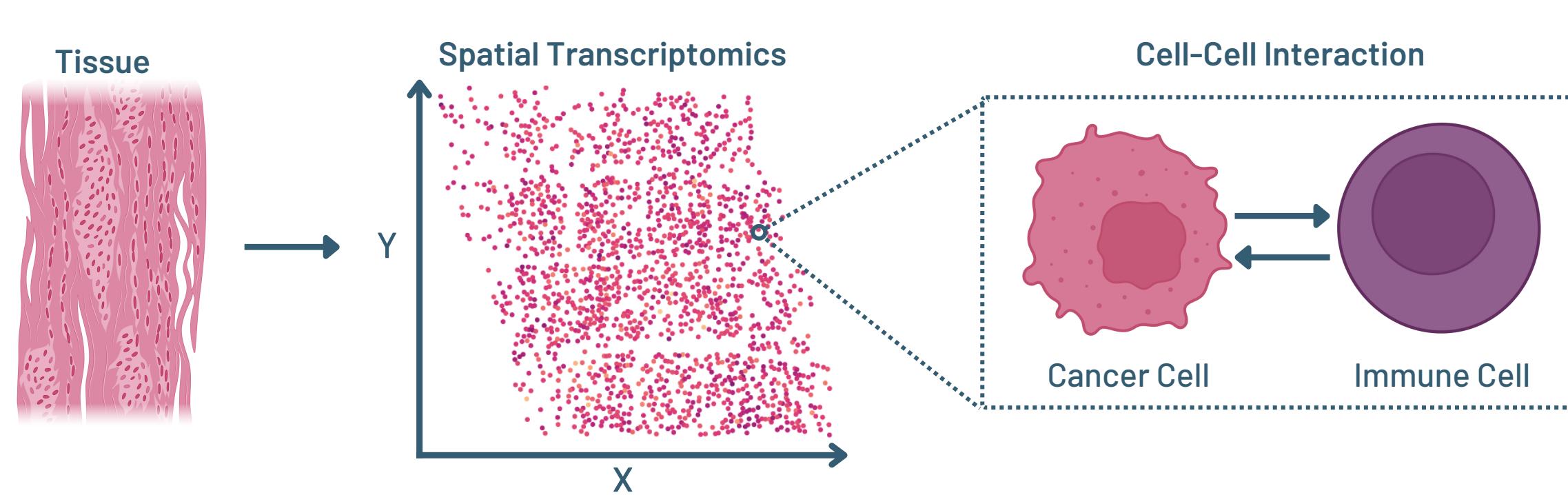
Xiuwei Zhang (Advisor)  
Georgia Institute of Technology



## 00 Motivation

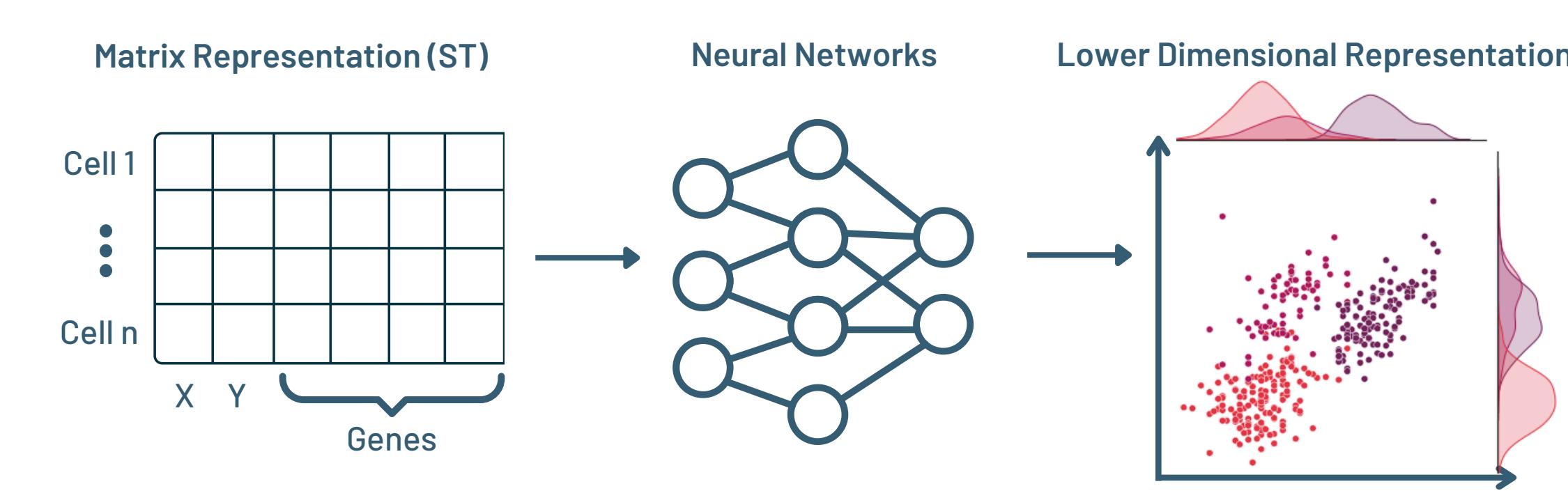
### 1. Why Spatial Transcriptomics?

With new genomic technologies, we are now generating the ability to slice cancerous tissue and identify the genes expressed in each single cell while also learning the spatial location of the cell. These technologies, known as *Spatial Transcriptomics* (ST), have myriad applications. For example, they offer an unprecedented look at the tumor microenvironment, revealing the infiltrating immune cells and their interactions with their cancerous counterparts. Thus, it is clear that we must utilize the power of Spatial Transcriptomics and elucidate cell-cell interactions (CCIs).



### 2. Why Deep Learning / GNNs?

With ST, we essentially obtain the expression of thousands of genes in cells of distinct spatial locations. This is often represented as vectors in high dimensional space. Many deep learning methods make use of this high dimensional data to discover higher order patterns, and ST is particularly amenable to such methods. Further, by representing the tissue as a graph (nodes are cells and edges represent spatial proximity) and using modern models (ie. Graph Neural Networks), we could learn lower manifold vector representations of each node, allowing us to make predictions regarding their interactions.



### 3. What is Needed?

Many computational methods now analyze the complex, high dimensional ST data for inferring these cell-cell interactions (CCIs). However, the ST community lacks a centralized ground truth to holistically evaluate these tools. Secondly, among the existing methods, many fail to incorporate spatial location properly, allowing for example, interactions between cells on opposite ends of a tissue slice. And, no current methods incorporate downstream gene regulatory information to aid their predictions. This has lead to a high number of false positive predictions, which must be improved. Thus, our objectives are as follows:

- (Aim 1) Benchmark the success and failures of current ST tools that infer CCIs to be accessible in a centralized database.
- (Aim 2) Refine CCI predictions with modern Deep Learning (DL) architectures that properly incorporate spatial location and downstream Gene Regulatory Network (GRN) information.

## 01

### Aim 1: Benchmarking CCI Methods

#### Objective:

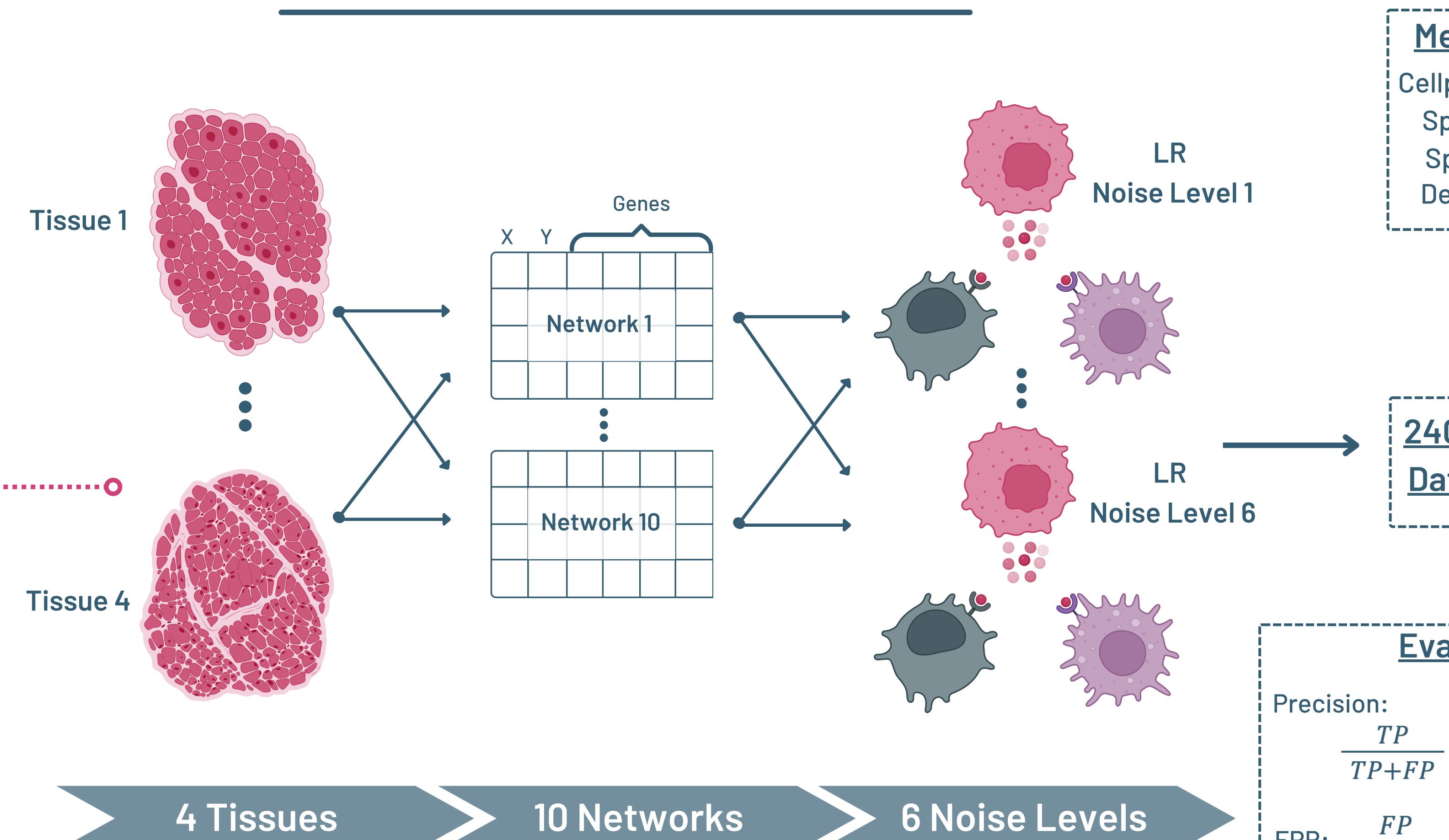
As the ST community lacks a common ground truth to compare contemporary tools, we propose to create a holistic and centralized database that evaluates all current ST-CCI methods on 240 simulated datasets along with real datasets (ie. SeqFISH+).

#### Methodology:

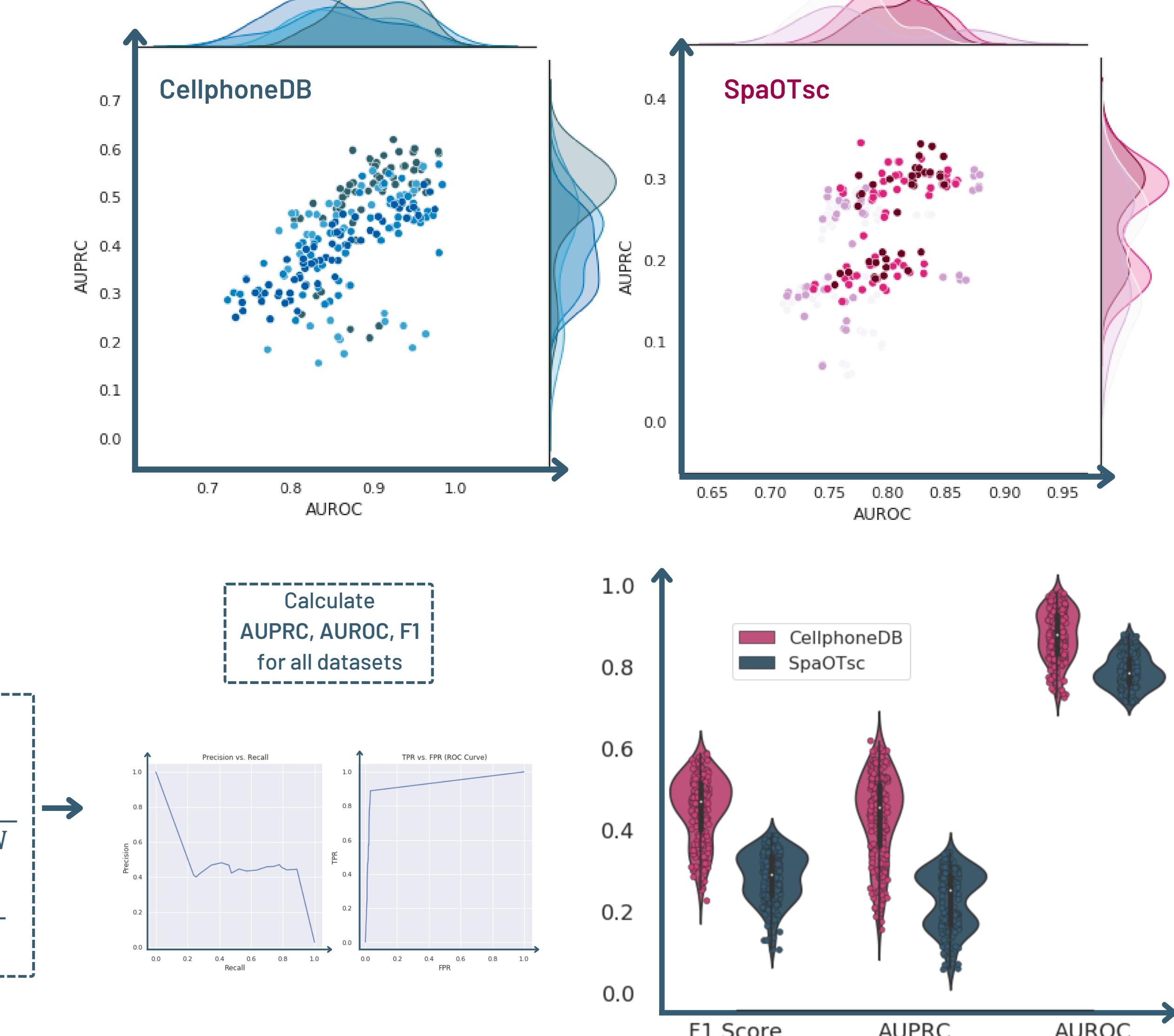
To simulate the datasets, we utilized an in-silico tissue simulator (MISTY) proposed by Tanevski et al. Four tissues were generated with two having five cell types and the remaining two having three cell types. Among each of the tissues, 10 networks were generated all containing 15 possible ligand-receptor pairs. Finally, to construct the ground truth ligand-receptor-pairs for each network, 6 noise levels were used.

Regarding the evaluation process, each method was compared to each of the datasets by metrics of precision, recall/TPR, and FPR. From these baseline metrics, we then visualized the performance in Precision Recall curves, ROC curves, and finally calculated AUPRC, AUROC, and f1 scores. On the final violin plot, each point represents a method's performance on a single dataset.

#### Simulating Datasets (MISTY)



#### CCI Method Evaluation/Results



## 02

### Aim 2: Refining CCI Inference with Subgraph Neural Networks

#### Objective:

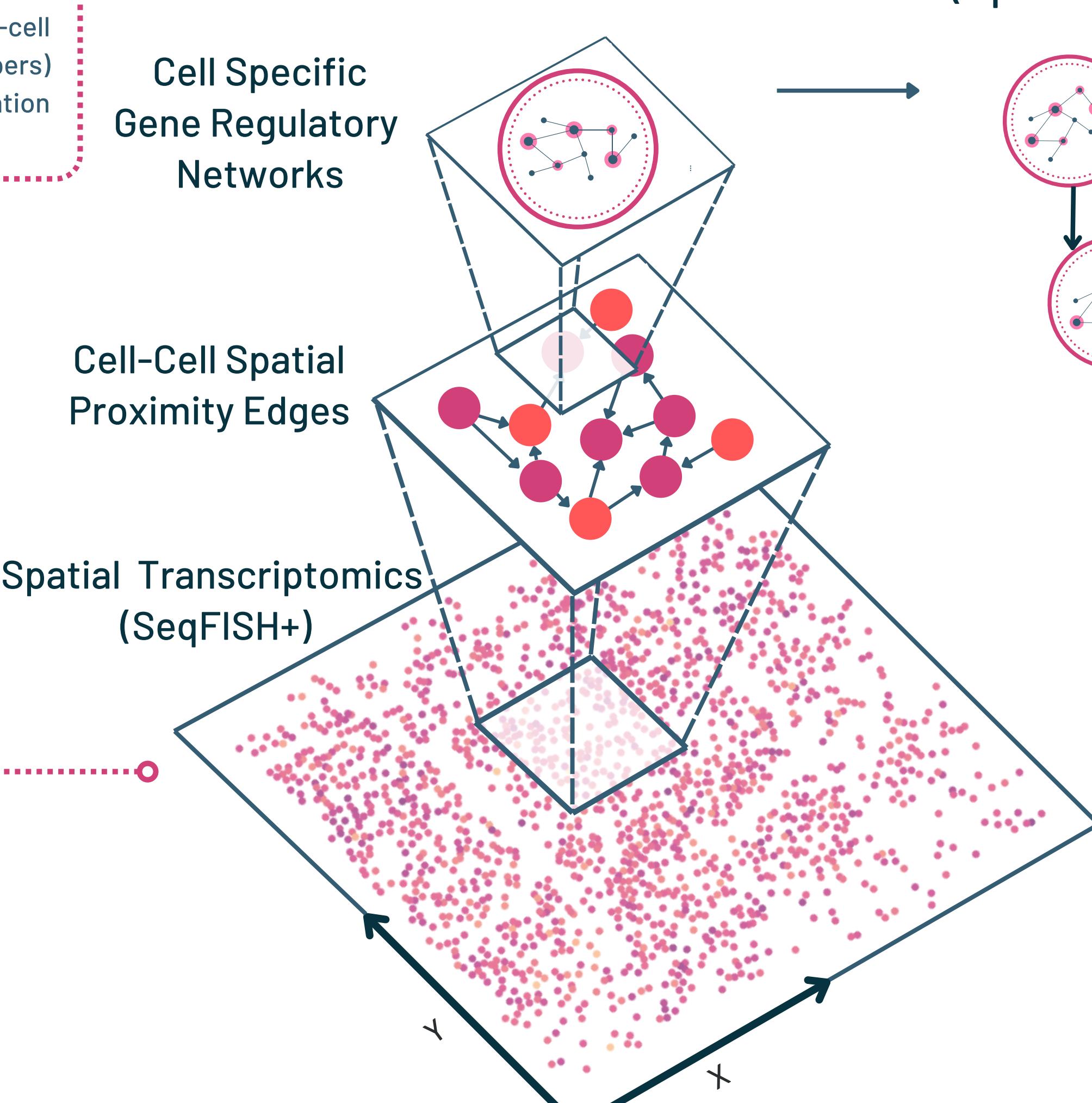
From the previous section, we can see that modern methods fall short in performance due to their high number of false positive predicted interactions. This may be because they fail to incorporate spatial location and downstream GRN information properly. Thus, we look to refine cell-cell interaction inference (cut down on the false positive numbers) by incorporating both spatial constraints and GRN information coupled with modern Graph Neural Network Architectures.

#### Graph Construction Methodology:

Traditionally, in our graph, we view each cell as a vertex where the edges between cells are based on spatial proximity. The edges contain the relationship of cells that are nearby to each other, thus allowing our inductive assumption that proximal cells have a higher chance of interaction. In this way, we incorporate the spatial information of our data. Now, in order to incorporate GRN information as well, we view each cell (vertex) as a subgraph of its underlying Gene Regulatory Network. Thus, our final graph (supergraph) consists of a collection of gene regulatory networks, where spatially close networks have genes that share additional edges.

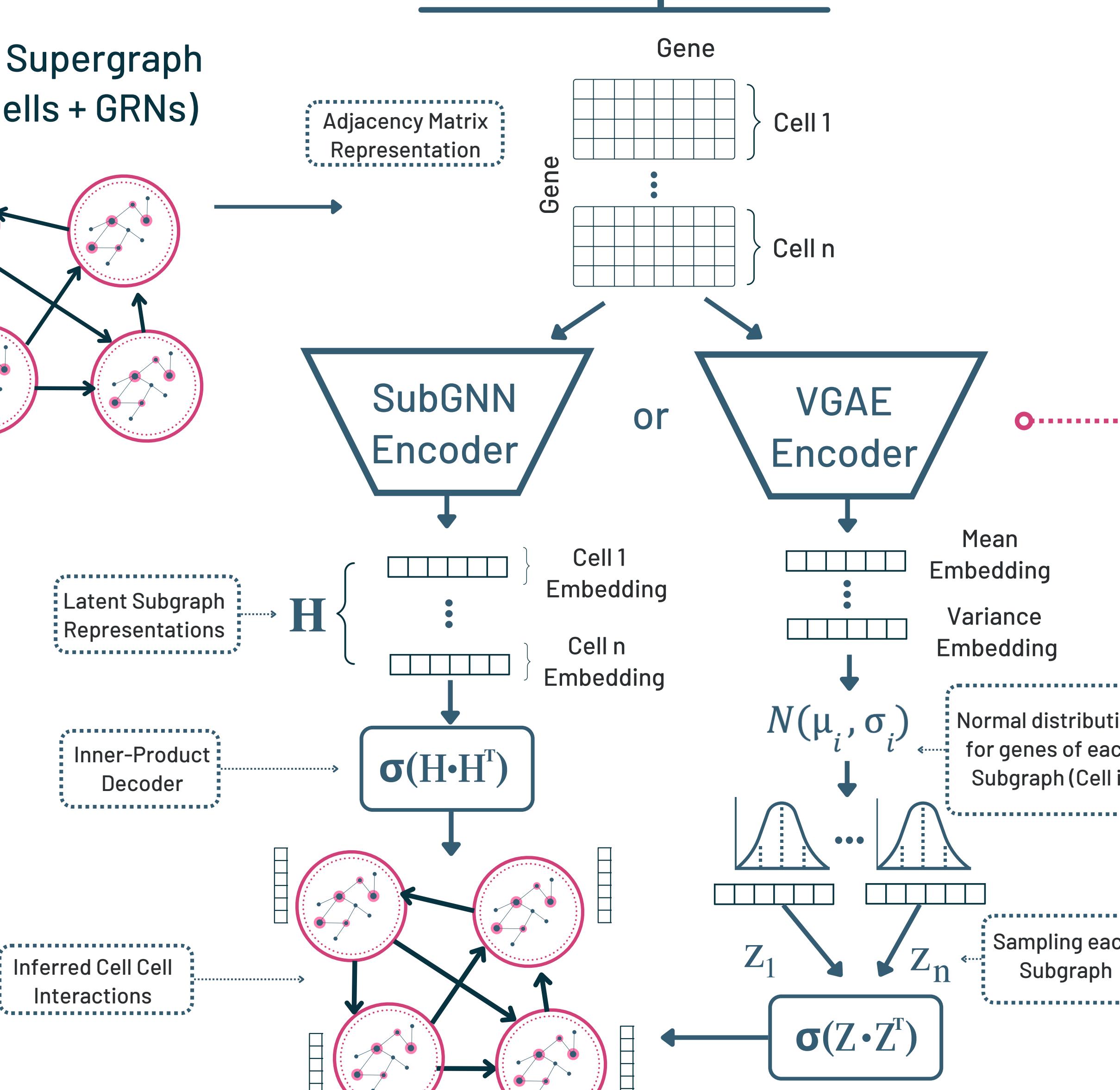
We tested our model on SeqFISH+ datasets and used CeSpGRN to infer GRNs for each individual cell. The genes of each GRN were connected to every gene of 5 neighboring GRNs in order to construct the supergraph. The adjacency matrix of this supergraph representation was used as the input to our model in order to obtain latent representations of each subgraph GRN. Each of these embeddings essentially represent a single cell in the dataset, thus simple cosine similarity was used to infer the potential edges that represent CCIs.

#### Graph Construction



#### Construct Supergraph (Spatial Cells + GRNs)

#### Model Pipeline



#### Model Architecture & Loss Methodology

##### Subgraph Neural Network (SubGNN)

Loss is calculated based on each subgraph embedding's respective position, neighborhood, and structure readout. These three embeddings are then concatenated for a final representation. The GNN layers follow the regular message passing scheme proposed by Kipf et al.

##### Graph Variational Autoencoder (GVAE)

Much like the canonical VAE, the loss is defined on the ability to reconstruct the original graph adjacency matrix as well as learn the original probability distribution. In this subgraph version, we learn a distribution for each subgraph (cell) of genes. The encoding layers can be formalized as such:

$$q(z_i | X) = N(z_i | \mu_i, \sigma_i)$$

And the decoding layers simply use an inner product mechanism:

$$p(X' | Z) = \langle z \cdot Z^T \rangle$$

Finally, the entire loss function can be summarized by two terms, one for reconstruction and one for similarity. For the reconstruction term, we use mean square error, and for learning the distributions (similarity), we use KL-divergence. Thus, given a graph with adjacency matrix  $X$ , the loss is defined as such:

$$L(X, X') = ||X - X'||^2 + D_{KL}(N(\mu_i, \sigma_i) || N(0, 1))$$

This model was able to achieve 0.85 precision for 0.97 recall on just the pretraining step, which is already an improvement to existing methodology, suggesting that our use of underlying GRNs does indeed improve and refine performance.

## 03

### Conclusions

#### Next Steps

##### Benchmarking:

- Evaluate methods on real ST data like SeqFISH+
- Publish our benchmarking paper so that the ST community can have access to our datasets and centralized ground truth

##### Refining CCI Inference (DL):

- Apply our various GNN architectures to all of our simulated datasets and properly benchmark its performance relative to existing methods
- Use GVAE for its potential generative applications (generate cells with specific GRNs based on spatial location)
- Try incorporating PPIs instead of GRNs as the underlying subgraph in our construction

#### References

1. Alsentzer, E., Finlayson, S., Li, M., & Zitnik, M. (2020). Subgraph neural networks. Advances in Neural Information Processing Systems, 33, 8017-8029.
2. Zhang, Z., Han, J., Song, L., & Zhang, X. (2022). Inferring cell-specific gene regulatory networks from single cell gene expression data. bioRxiv.
3. Tanevski, J., Flores, R. O. R., Gabor, A., Schapiro, D., & Saez-Rodriguez, J. (2021). Explainable multi-view framework for dissecting intercellular signaling from highly multiplexed spatial data. BioRxiv, 2020-05.
4. Li, R., & Yang, X. (2022). De novo reconstruction of cell interaction landscapes from single-cell spatial transcriptome data with DeepLink. Genome Biology, 23(1), 1-24.
5. Efremova, M., Vento-Tormo, M., Teichmann, S. A., & Vento-Tormo, R. CellPhoneDB v2. 0: Inferring cell-cell communication from combined expression of multi-subunit receptor-ligand complexes. doi: 10.680926.
6. Shao, X., Li, C., Yang, H., Lu, X., Liao, J., Jian, J., ... & Fan, X. (2022). Knowledge-graph-based cell-cell communication inference for spatially resolved transcriptomic data with SpaTalk. bioRxiv.
7. Cang, Z., & Nie, Q. (2020). Inferring spatial and signaling relationships between cells from single cell transcriptomic data. Nature Communications, 11(1), 1-13.

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