

Spatial Transcriptomics and CellNEST: Mapping Cellular Conversations

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In 2020, Nature Methods announced spatially resolved transcriptomics as its Method of the Year. Since then, rapid advancements in spatial transcriptomic technologies have sparked a wave of computational tools to interpret and extract meaningful insights from these datasets. But what exactly makes spatial transcriptomics so powerful?

Unlike regular transcriptomics, which tells us *which* genes are active, spatial transcriptomics tells us both *which* genes are active and *where* they are active spatially within the tissue. Genes act as an instruction manual for cells, and which ‘instructions’ are being read determines how the cell behaves. But cells don’t function in isolation – they work together and communicate to coordinate various cell activities crucial to their function, growth, and survival. When cells fail to communicate in a normal, regulated way, diseases can emerge.

Thus, cell-cell communication is key for understanding biological processes underlying both healthy and diseased states. So how can we detect these hidden conversations?

Communication Through Ligand-Receptor Pairs

One of the ways that cells talk to each other is through ligand-receptor pairs (LRPs). Think of ligands as ‘keys’ produced by the sender cell, which bind to the corresponding receptors – the ‘locks’ – on a neighbouring receiver cell, and consequently triggers a downstream response within the receiver cell. This simple mechanism allows cells to communicate with one another and is responsible for regulating a variety of biological processes such as tissue growth and immune activation.

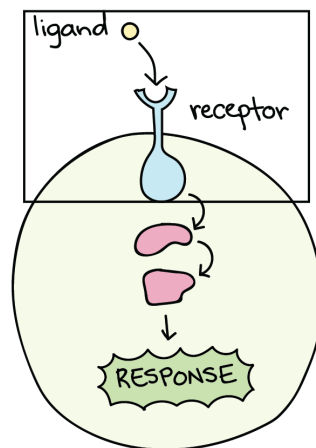


Figure 1. Schematic showing a ligand-receptor interaction, along with its downstream response in the receiver cell (*Khan Academy*).

Spatial transcriptomics data includes the expression levels for these ligands and receptors, along with their spatial location. This means that we can map who might be talking to whom – but making sense of thousands of potential interactions is hard without computational tools.

Meet CellNEST

CellNEST (Cellular Neural Networks on Spatial Transcriptomics), developed by researchers at the Schwartz Lab from Princess Margaret Cancer Centre (UHN), the University of Toronto's Department of Medical Biophysics, and the Vector Institute for Artificial Intelligence, is one such tool. Leveraging deep learning methods, the tool enables detection of hidden patterns of communication by integrating both spatial context and gene expression levels in its model.

CellNEST uses a graph attention network (GAT) model– a type of graph neural network (GNN). GNNs are neural networks that operate on data represented as graphs, where vertices represent entities and edges represent the relationships between them.

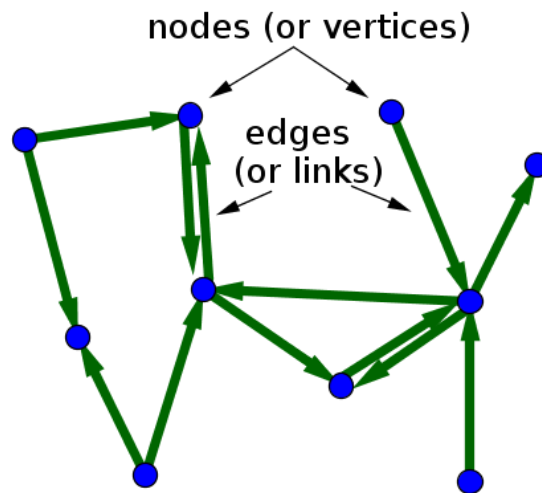


Figure 2. A graph with 6 vertices/nodes and 6 edges (*Directed Graph Definition - Math Insight*). Note the figure above shows a directed graph (i.e. the edges have a specific direction, vs edges in an undirected graph do not have a direction). CellNEST uses directed graphs in its model.

CellNEST generates graph-structured data using the input dataset, where cells or spots on the tissue are represented as vertices, and edges denote relationships such as potential communication. The GAT model is trained using an unsupervised contrastive learning approach called deep graph infomax (DGI). DGI compares the graph created using the input data to a ‘corrupted’ graph of randomly shuffled and permuted vertices and edges, to identify which communication patterns are most relevant to capturing the context of the tissue sample. CellNEST then uses this information to assign ‘attention scores’ to each edge in the graph, reflecting the strength of communication between the connected vertices.

CellNEST uses the top scoring edges to create a visualization of the tissue showing uniquely colored communities called ‘components’ representing distinctly communicating cells or spots. Highly communicating regions of the tissue are shown as densely colored spots on the graph while LRPs between spots are depicted as arrows of varying levels of thickness determined using attention scores. Users can also view top LRPs and how much they contribute to each component in a histogram. Additionally, CellNEST also allows the detection of relay-networks (i.e. communication between multiple cells through a consecutive sequence of ligand-receptor-ligand-receptor interactions).

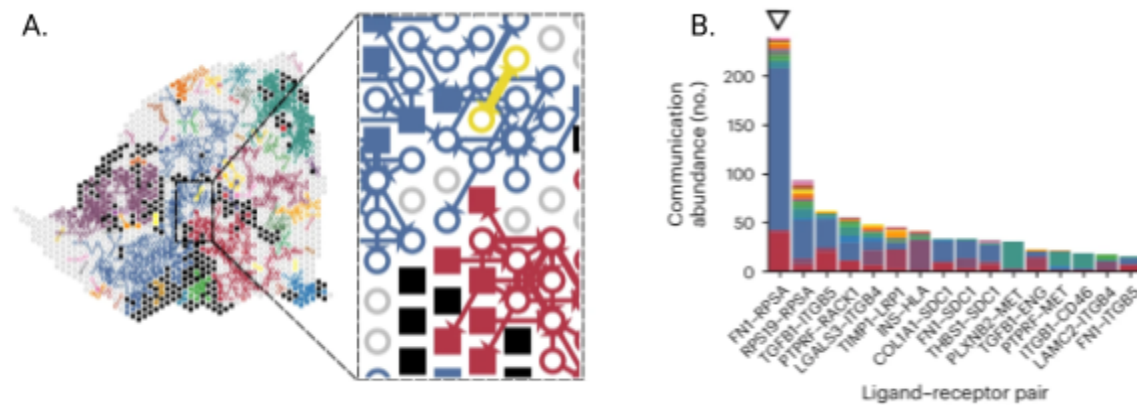


Figure 3. Example outputs generated by CellNEST. (A) An example output graph generated by CellNEST showing ligand-receptor interactions between tumor-annotated spots (filled squares) and stroma spots (open circles), colored uniquely by the component they belong to (Zohora et al.). (B) An example of a histogram generated by CellNEST, showing the frequency of ligand-receptor interactions making up the top 20% highest scoring edges, with the bars colored correspondingly with the components they are found in (Zohora et al.).

Key Features

Here are a few key features of CellNEST:

- Flexible options for visualization, including cell-type annotations and spatial mapping of specific ligand-receptor interactions
- Supports detection of cell-cell communication, including relay-networks, in both 2D and 3D spatial transcriptomic datasets
- Adjustable parameters, giving users flexibility in how they apply the tool
- Compatible with multiple spatial transcriptomic technologies, including MERFISH, Visium, and Visium HD
- Choice of using the provided ligand-receptor databases or another database of the user’s choice

Implications and Future Directions

CellNEST has a broad range of potential applications. For example, it can help uncover how cell-cell communication supports and/or drives healthy and diseased states, particularly relevant for cancers, which are often genetically heterogenous and shaped by complex cell-cell interactions. Insights into

communication networks may also be associated with certain clinical outcomes including trends in survival and response to treatment.

Future developments of CellNEST may include integration of other spatial-omic technologies to improve accuracy and validation of detected ligand-receptor interactions. It will also be exciting to see how CellNEST performs on different tissue contexts and how creative applications of the model reveal new biological insights from spatial transcriptomics data.

References

Directed Graph Definition - Math Insight. https://mathinsight.org/definition/directed_graph. Accessed 21 Sep. 2025.

Khan Academy.

<https://www.khanacademy.org/science/biology/cell-signaling/mechanisms-of-cell-signaling/a/signal-perception>. Accessed 20 Sep. 2025.

Zohora, Fatema Tuz, et al. “CellNEST Reveals Cell–Cell Relay Networks Using Attention Mechanisms on Spatial Transcriptomics.” *Nature Methods*, vol. 22, no. 7, Jul. 2025, pp. 1505–19. *www.nature.com*, <https://doi.org/10.1038/s41592-025-02721-3>.