

STGNET: extending panel coverage in imaging-based spatial transcriptomics using deep generative adversarial networks

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Abstract

Imaging-based spatial transcriptomics (ST) technologies offer unparalleled resolution for mapping gene expression within intact tissues but are fundamentally constrained by the limited size of their gene panels. This restriction hinders comprehensive biological discovery by omitting potentially crucial genes from analysis. To overcome this limitation, we introduce STGNET, a deep learning framework that extends gene panel coverage by integrating generative adversarial networks (GANs) with graph neural networks. STGNET employs a multi-stage GAN to learn the global transcriptomic distribution from single-cell RNA sequencing data, followed by a spatially aware graph convolutional network that refines imputations by modeling both physical cell proximity and transcriptional similarity. We rigorously benchmarked STGNET against seven state-of-the-art methods across nine diverse ST datasets. STGNET consistently achieved superior performance, demonstrating enhanced accuracy in gene imputation, and exceptional preservation of cellular topology. We further showcase its biological utility by accurately reconstructing developmental marker patterns in mouse embryogenesis, revealing a novel transitional cell state in breast cancer progression, and uncovering extensive, previously obscured cell–cell communication networks in the mouse brain. STGNET provides a powerful and robust solution for unlocking the full potential of targeted ST assays, thereby enabling deeper and more comprehensive spatial biology. STGNET is freely accessible at <https://github.com/wuyuanwuhui/STGNET>.

Keywords imaging-based spatial transcriptomics, generative adversarial networks, graph convolutional network, gene panel imputation

Introduction

The functional architecture of complex tissues is fundamentally governed by the precise spatial organization of and communication between their constituent cells, a critical dimension obliterated by dissociative single-cell RNA sequencing (scRNA-seq) [1, 2]. The emergence of spatial transcriptomics (ST) has bridged this gap, offering an unprecedented window into the molecular geography of tissues [3, 4]. Among these technologies, imaging-based *in situ* profiling methods—such as MERFISH [5], seqFISH+ [6], and the

commercial Xenium [7] and CosMx platforms [8]—have set a new standard for sensitivity and spatial resolution, enabling the mapping of hundreds to thousands of genes at subcellular precision. Despite their transformative potential, these powerful methods are intrinsically constrained by a fundamental limitation: their reliance on a predefined gene panel, which severely limits transcriptome-wide discovery and can introduce bias by requiring a priori gene selection [9]. This physical bound, imposed by optical crowding, spectral overlap, and lengthy imaging cycles, creates a critical bottleneck [10].

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It forces a trade-off between resolution and discovery, potentially obscuring novel biomarkers, unanticipated cellular states, and the completeness of biological pathways, thereby limiting the utility of these rich datasets for hypothesis-free exploration [11, 12].

In response to this panel limitation, several computational strategies have been employed, yet they are fundamentally constrained by their reliance on single-cell RNA sequencing reference atlases and are often ill-suited for the specific task of high-resolution panel extension. A prevalent paradigm, used by tools like Tangram [13], SpaGE [14], and deep learning-based methods like gimVI [15] and stPlus [16], relies on aligning spatial and single-cell data through a limited set of shared genes. This approach is inherently susceptible to technical confounders like batch effects and dropout events, which can distort the mapping and compromise the accuracy of imputed expression values. Moreover, the entire inference is bottlenecked by the number of shared genes, limiting the effective prediction of a broader transcriptome. More recently, diffusion-based models like SpatialScope [17] and stDiff [18] have emerged, framing data enhancement as a generative process. While powerful, these models primarily learn from the correlation structure of gene expression within dissociated scRNA-seq cells, treating expression profiles as the primary determinant of cell state. Consequently, they fail to explicitly and effectively leverage the rich, continuous spatial context—such as cellular neighborhoods and morphological regions—that is uniquely present in imaging-based ST data [19]. This oversight prevents them from capturing the critical spatial expression patterns and gradients that are essential for accurate, context-aware gene imputation *in situ*, thus creating a pronounced methodological gap for a dedicated solution.

To address these limitations, we introduce STGNET, a deep learning framework architected around two core principles: robust generative modeling and direct spatial integration. STGNET's first key innovation is a multi-stage generative adversarial networks (GANs) process, stabilized by the Wasserstein GAN with gradient penalty (WGAN-GP) [20], which is trained to learn the underlying distribution of gene expression from a scRNA-seq reference. This approach focuses on capturing the fundamental structure of the transcriptome, making it more resilient to the technical noise and dropout events that plague individual cells in reference data. The second, and crucial, innovation is the direct incorporation of spatial context through a multi-view graph convolutional network (GCN). This GCN simultaneously models the tissue architecture using a spatial graph of physical cell adjacencies and a feature graph of transcriptional similarities. An integrated attention mechanism then dynamically fuses these two views, allowing the model to adaptively weight the importance of a cell's physical neighborhood versus its transcriptional identity for each imputation event. This ensures that the final predicted expression values are not only transcriptionally plausible but also spatially coherent within the tissue microenvironment.

We rigorously evaluate its performance against state-of-the-art methods across nine public datasets, showing that STGNET robustly imputes missing gene expression with high fidelity to held-out ground truth data. We further validate that the imputed data preserves critical spatial patterns and enhances downstream biological analyses, including the identification of spatially variable genes and the inference of developmental trajectories. By providing a method that directly addresses the core limitations of existing approaches, STGNET establishes itself as a powerful tool for unlocking the full potential of spatially resolved transcriptomics.

Materials and methods

Employing multiple generative adversarial networks to facilitate initial spatial transcriptomics imputation

STGNET is a deep learning framework that integrates GANs and GNNs to impute and extend gene expression profiles in ST data. STGNET employs multiple GANs to facilitate initial ST imputation. This phase operates in three sequential, adversarial training steps to ensure robustness: first, learning a global reference distribution from scRNA-seq data; second, imputing missing values in scRNA-seq data using a masked learning approach; and third, transferring and refining these imputations within the spatial context of ST data. An overview of the architecture is provided in [Supplementary Figs S1–S3](#).

Adversarial training with Wasserstein generative adversarial network and gradient penalty

To ensure stable training and mitigate mode collapse common in standard GANs, all generative components in STGNET are built upon the Wasserstein GAN with Gradient Penalty (WGAN-GP) framework. The WGAN objective minimizes the Earth-Mover (Wasserstein-1) distance, which provides a more meaningful learning gradient than the Jensen–Shannon divergence used in original GANs.

The general objective function for a generator G and a discriminator D is:

$$\min_G \max_{D \in \mathcal{D}} \mathbb{E}_{x \sim p_{\text{data}}} [D(x)] - \mathbb{E}_{z \sim p_z(z)} [D(G(z))] + \lambda \cdot (\|\nabla_{\hat{x}} D(\hat{x})\|_2 - 1)^2, \quad (1)$$

where $\mathcal{D}$ is the set of 1-Lipschitz functions, p_{data} is the real data distribution, and p_z is the noise distribution. The final term is the gradient penalty, where $\hat{x}$ is a random interpolation between real and generated samples ($\hat{x} = \varepsilon x_{\text{real}} + (1 - \varepsilon) x_{\text{fake}}$, $\varepsilon \sim U[0, 1]$), and λ is a hyperparameter. This penalty enforces the 1-Lipschitz constraint more reliably than weight clipping.

Learning the global single-cell RNA sequencing distribution

The objective of the first phase is to learn a robust generator G_X that captures the underlying distribution of the global scRNA-seq reference atlas. The input scRNA-seq data matrix $X \in \mathbb{R}^{N \times M}$ (with N cells and M genes) is normalized (TPM followed by $\log_1 p$ transformation). Generator G_X takes random noise z and, optionally, cell-type labels as input to produce a synthetic expression profile $\tilde{X} = G_X(z)$. The discriminator D_X is trained to distinguish between samples from the real data distribution $p_{\text{data}}(X)$ and the generated distribution $p_g(\tilde{X})$. The loss for this phase is defined as:

$$\mathcal{L}_X = \min_{G_X} \max_{D_X} [\mathbb{E}_{X \sim p_{\text{data}}} [D_X(X)] - \mathbb{E}_{z \sim p_z(z)} [D_X(G_X(z))]]. \quad (2)$$

A well-trained G_X provides a foundational model of plausible transcriptomic states, which bootstraps the subsequent imputation phases.

Single-cell RNA sequencing data imputation

The objective of the second phase is to train a generator G_{SC} that can accurately impute missing values (dropouts) in scRNA-seq data. This phase employs a U-Net-based generator G_{SC} (see [Supplementary Information](#)). The model learns in a self-supervised manner by artificially masking a portion of the genes in a real scRNA-seq cell profile x , creating a masked vector x_{masked} and a corresponding binary mask

matrix m . The generator takes the tuple $(x_{\text{masked}}, m, z)$ as input and outputs an imputed vector $\tilde{x}_{\text{SC}}$. The adversarial loss for this phase is:

$$\mathcal{L}_{\text{SC}} = \min_{G_{\text{SC}}} \max_{D_{\text{SC}}} [\mathbb{E}_{x \sim p_{\text{data}}} [D_{\text{SC}}(x)] - \mathbb{E}_{x, m} [D_{\text{SC}}(G_{\text{SC}}(x_{\text{masked}}, m, z))]] \quad (3)$$

This forces G_{SC} to generate imputations that are indistinguishable from non-masked, real data, thereby learning to correct for technical noise and dropouts. After the training process converges, G_{SC} is used for the imputation of scRNA-seq data.

Spatial-aware imputation for spatial transcriptomics data

The objective of the third phase is to adapt the imputation model to the spatial context of ST data, training a final generator G_{ST} . This is the core spatial integration phase. The input is ST data, which includes the gene expression matrix, noise, mask matrix, cell type, and the spatial coordinates of each spot/cell. It takes the similar inputs as G_{SC} (masked expression, noise, mask matrix, and cell type) but now also propagates information across the coordinates. By introducing spatial coordinates, the data generated by similar nodes becomes more similar. The discriminator D_{ST} is trained to discriminate between data generated by G_{ST} and data generated by the well-trained scRNA-seq imputer G_{SC} . This aligns the ST imputations with the global transcriptomic distribution learned from scRNA-seq. Additionally, a mean squared error (MSE) loss between the generated data and the real, non-masked portions of the ST data is added to ensure fidelity. The composite loss function for this phase is:

$$\mathcal{L}_{\text{ST}} = \min_{G_{\text{ST}}} \max_{D_{\text{ST}}} [\mathbb{E}[D_{\text{ST}}(G_{\text{SC}}(\cdot))] - \mathbb{E}[D_{\text{ST}}(G_{\text{ST}}(\cdot))]] + \alpha \cdot \text{MSE}(x_{\text{real}}, \tilde{x}_{\text{ST}}), \quad (4)$$

where α is a hyperparameter balancing the adversarial and reconstruction losses.

Cell type label handling

When explicit cell type labels are unavailable for the scRNA-seq or ST data, STGNET performs unsupervised clustering (e.g. Leiden clustering) on the expression data to assign provisional labels, which are then used as conditional inputs to the generators.

Integrating spatial information to refine spatial transcriptomics imputation

Building upon the initial imputation from the multiple GANs phase, STGNET employs a sophisticated graph-based framework to further enhance imputation quality by explicitly modeling the spatial dependencies inherent in transcriptomic data. This refinement phase operates on the fundamental biological principle that spatial proximity often correlates with functional similarity, driven by shared microenvironments and cell-cell communication. This stage is divided into three parts: first, STGNET constructs adjacency graphs based on spatial coordinates, and gene expression information, respectively, to fully integrate tissue structural information. Second, an attention mechanism is used to dynamically merge these two types of features for integration. Third, a comprehensive optimization objective is employed to achieve precise imputation of ST data.

Multi-view graph construction

To capture both physical adjacency and transcriptional relationships, we construct two complementary graph representations of the ST data.

The spatial graph $\mathcal{G}_s = (\mathbf{A}_s, \mathbf{X})$ encodes physical tissue organization, where $\mathbf{A}_s \in \mathbb{R}^{N \times N}$ represents the spatial adjacency matrix, and $\mathbf{X} \in \mathbb{R}^{N \times M}$ denotes the imputed gene expression matrix. Spatial connectivity is determined by Euclidean distance between spots:

$$A_s^{ij} = \begin{cases} 1, & \text{if } d(\mathbf{c}_i, \mathbf{c}_j) \leq r \\ 0, & \text{otherwise,} \end{cases} \quad (5)$$

where $d(\mathbf{c}_i, \mathbf{c}_j)$ represents the Euclidean distance between spatial coordinates of spots i and j , and r is a predefined radius threshold.

The feature graph $\mathcal{G}_f = (\mathbf{A}_f, \mathbf{X})$ captures transcriptional similarity using cosine similarity in gene expression space:

$$\text{sim}(\mathbf{x}_i, \mathbf{x}_j) = \frac{\mathbf{x}_i \cdot \mathbf{x}_j}{\|\mathbf{x}_i\| \|\mathbf{x}_j\|}. \quad (6)$$

The feature adjacency matrix $\mathbf{A}_f$ is constructed by connecting each spot to its k -nearest neighbors based on transcriptional similarity.

Multi-view graph convolutional encoder

We employ a multi-view GCN to extract and integrate information from both spatial and feature perspectives. The spatial convolution operates as:

$$\mathbf{H}_s^{(l+1)} = \text{ReLU} \left(\tilde{\mathbf{D}}_s^{-\frac{1}{2}} \tilde{\mathbf{A}}_s \tilde{\mathbf{D}}_s^{-\frac{1}{2}} \mathbf{H}_s^{(l)} \mathbf{W}_s^{(l)} \right), \quad (7)$$

where $\tilde{\mathbf{A}}_s = \mathbf{A}_s + \mathbf{I}$, $\tilde{\mathbf{D}}_s$ is the corresponding degree matrix, $\mathbf{W}_s^{(l)}$ are trainable weights, and $\mathbf{H}_s^{(0)} = \mathbf{X}$.

Similarly, the feature convolution is defined as:

$$\mathbf{H}_f^{(l+1)} = \text{ReLU} \left(\tilde{\mathbf{D}}_f^{-\frac{1}{2}} \tilde{\mathbf{A}}_f \tilde{\mathbf{D}}_f^{-\frac{1}{2}} \mathbf{H}_f^{(l)} \mathbf{W}_f^{(l)} \right), \quad (8)$$

with $\tilde{\mathbf{A}}_f = \mathbf{A}_f + \mathbf{I}$ and $\mathbf{H}_f^{(0)} = \mathbf{X}$.

Attention-based feature fusion

To adaptively balance contributions from spatial and transcriptional views, we employ an attention mechanism:

$$\mathbf{a}_s = \text{softmax}(\mathbf{w}_s \cdot \tanh(\mathbf{W}\mathbf{H}_s + \mathbf{b})), \quad (9)$$

$$\mathbf{a}_f = \text{softmax}(\mathbf{w}_f \cdot \tanh(\mathbf{W}\mathbf{H}_f + \mathbf{b})). \quad (10)$$

The final integrated representation is obtained through:

$$\mathbf{H} = \mathbf{F}(\mathbf{a}_s \cdot \mathbf{H}_s + \mathbf{a}_f \cdot \mathbf{H}_f), \quad (11)$$

where $\mathbf{F}$ represents a linear transformation layer that captures highly variable features of the latent representations.

Zero-inflated negative binomial decoder

To accurately model the characteristics of spatial transcriptomic data, we employ a zero-inflated negative binomial (ZINB) decoder that

accounts for over-dispersion and excess zeros:

$$P_{\text{ZINB}}(x_{ij}) = \pi_{ij}\delta_0(x_{ij}) + (1 - \pi_{ij})\text{NB}(x_{ij} \mid \mu_{ij}, \theta_{ij}), \quad (12)$$

$$\text{NB}(x_{ij} \mid \mu_{ij}, \theta_{ij}) = \frac{\Gamma(x_{ij} + \theta_{ij})}{\Gamma(x_{ij} + 1)\Gamma(\theta_{ij})} \left(\frac{\theta_{ij}}{\theta_{ij} + \mu_{ij}} \right)^{\theta_{ij}} \left(\frac{\mu_{ij}}{\theta_{ij} + \mu_{ij}} \right)^{x_{ij}}. \quad (13)$$

The reconstruction loss is formulated as the negative log-likelihood:

$$\mathcal{L}_{\text{ZINB}} = -\frac{1}{N \cdot M} \sum_{i=1}^N \sum_{j=1}^M \ln P_{\text{ZINB}}(x_{ij}). \quad (14)$$

Spatial regularization constraints

To preserve spatial neighborhood relationships in the latent space, we impose a spatial regularization constraint:

$$\mathcal{L}_{\text{reg}} = -\sum_{i=1}^N \left(\sum_{j \in \mathcal{N}_i} \log(\sigma(C_{ij})) + \sum_{k \notin \mathcal{N}_i} \log(1 - \sigma(C_{ik})) \right), \quad (15)$$

where C_{ij} represents the cosine similarity between latent representations of spots i and j , and $\mathcal{N}_i$ denotes the set of spatial neighbors for spot i .

Joint optimization framework

The complete optimization objective integrates all components through a weighted combination:

$$\mathcal{L} = \eta \mathcal{L}_{\text{ZINB}} + \beta \mathcal{L}_{\text{reg}} + \gamma \mathcal{L}_{\text{KL}}, \quad (16)$$

where η , β , and γ are balancing coefficients that control the contributions of reconstruction accuracy, spatial coherence, and distributional consistency with the original data, respectively. This multi-objective optimization ensures that the refined imputations are both biologically plausible and spatially coherent.

Validation datasets

To ensure a comprehensive evaluation of STGNET's performance, we curated a diverse collection of nine publicly available ST datasets. These datasets were generated using a variety of experimental platforms (e.g. MERFISH, seqFISH, and Xenium) and encompass multiple tissue and organ types, providing a robust testbed that reflects the heterogeneity of real-world data. The selected datasets exhibit considerable variation in the number of cells/spots and genes, as detailed in Table 1.

A subset of these ST datasets (MERFISH, seqFISH, and Xenium) includes authoritative cell type annotations. For the remaining datasets lacking such annotations, we first performed unsupervised clustering on the paired single-cell RNA sequencing (scRNA-seq) reference data using the Leiden algorithm [21] to assign provisional cell type labels. These labels were then transferred to the ST data using the robust deconvolution method implemented in SpatialScope [17], ensuring consistent cell type information across all datasets for downstream evaluation.

Benchmarking and baseline methods

We benchmarked STGNET against seven state-of-the-art methods for spatial data imputation and integration:

- **Tangram** [13]: A mapping-based method that aligns scRNA-seq data to spatial data.
- **gimVI** [15]: A deep generative model based on a variational autoencoder for joint analysis of ST and scRNA-seq data.
- **stPlus** [16]: A method that uses reference scRNA-seq and an autoencoder for joint embedding to enhance ST data.
- **SpaGE** [14]: A machine learning approach that integrates scRNA-seq and ST data to predict unmeasured genes.
- **uniPort** [33]: A unified framework for the integration and imputation of single-cell and spatial data.
- **SpatialScope** [17]: A method employing diffusion-based models to reference scRNA-seq data for ST data completion.
- **stDiff** [18]: A recent diffusion-model-based approach for enhancing ST data.

For all baseline methods, data preprocessing steps—including normalization and scaling—were performed in strict accordance with the instructions and source code provided by the original authors. Detailed source code references and parameter settings are documented in [Supplementary Materials](#).

Performance metrics

We conducted a multi-faceted evaluation of imputation performance from both cellular and genic perspectives.

Cellular-level evaluation

To assess the ability of imputed data to preserve biological identity and cellular relationships, we evaluated the consistency of cell clustering results between the imputed and ground-truth ST data. We employed four established clustering metrics [34]:

- **Adjusted Rand Index (ARI)**: Measures the similarity between two data clusterings, corrected for chance.
- **Adjusted Mutual Information (AMI)**: Quantifies the mutual information between two clusterings, adjusted for chance.
- **Normalized Mutual Information (NMI)**: A normalization of the Mutual Information score to scale the results between 0 (no mutual information) and 1 (perfect correlation).
- **Homogeneity (Homo)**: Measures whether each cluster contains only members of a single class.

Higher values for all four metrics indicate better clustering consistency and thus superior preservation of cellular states.

Genic-level evaluation

To evaluate the accuracy of gene expression prediction, we employed a five-fold cross-validation strategy, holding out a portion of genes as ground truth. We used four complementary metrics:

- **Spearman's rank correlation coefficient (SPCC)**: A non-parametric measure of the monotonic relationship between the predicted and true expression values for a gene across all cells. A higher SPCC indicates better preservation of expression rankings.

$$\text{SPCC}(i) = 1 - \frac{6 \sum_{j=1}^n d_{ij}^2}{n(n^2 - 1)}, \quad d_{ij} = \text{rank}(T_{ij}) - \text{rank}(P_{ij}). \quad (17)$$

- **Structural similarity index measure (SSIM)**: Assesses the perceptual similarity between the spatial expression patterns of predicted and true values for a gene. A higher SSIM indicates better

Table 1 Summary of the nine validation dataset pairs

Tissue	scRNA-seq data				Spatial data			
	Cell num	Gene num	Reference	Technique	Cell num	Gene num	Reference	Technique
Mouse embryonic	9991	16 477	[22]	Microwell	175	42	[23]	seqFish
Mouse primary motor cortex	14 249	34 041	[24]	AllenVISp	5551	247	[25]	MERFISH
Mouse osteosarcoma	9234	19 098	[26]	10× Genomics	645	915	[27]	MERFISH
Mouse gastrulation	4651	19 103	[28]	10× Genomics	8425	347	[28]	seqFish
Primary visual cortex	14 249	34 041	[23]	AllenVISp	1154	42	[29]	ExSeq
Somatosensory cortex	5613	30 527	[24]	AllenSSp	3405	33	[30]	osmFISH
Primary visual cortex	14 249	34 041	[24]	AllenVISp	2399	244	[31]	MERFISH
Drosophila Embryo	1297	8924	[32]	Drop-seq	3039	84	[32]	FISH
Human breast cancer	18 783	16 096	[7]	scFFPE-seq	19 976	306	[7]	Xenium

preservation of spatial structure.

$$\text{SSIM}(i) = \frac{(2\mu_{P_i}\mu_{T_i} + C_1)(2\text{conv}(P_i, T_i) + C_2)}{(\mu_{P_i}^2 + \mu_{T_i}^2 + C_1)(\sigma_{P_i}^2 + \sigma_{T_i}^2 + C_2)}. \quad (18)$$

- **Root mean square error (RMSE):** Measures the magnitude of the absolute differences between predicted and true expression values. A lower RMSE indicates higher numerical accuracy.

$$\text{RMSE}(i) = \sqrt{\frac{1}{n} \sum_{j=1}^n (P_{ij} - T_{ij})^2}. \quad (19)$$

- **Jensen–Shannon divergence (JS):** A symmetric and bounded measure of the similarity between the probability distributions of predicted and true expression values for a gene. A lower JS divergence indicates greater distributional similarity.

$$\text{JS}(i) = \frac{1}{2} \text{KL}(\phi_i(P)|M_i) + \frac{1}{2} \text{KL}(\phi_i(T)|M_i),$$

$$\text{where } M_i = \frac{\phi_i(P) + \phi_i(T)}{2}, \quad \phi_{ij}(X) = \frac{X_{ij}}{\sum_{k=1}^n X_{ik}}. \quad (20)$$

The Kullback–Leibler (KL) divergence is given by:

$$\text{KL}(P_i|Q_i) = \sum_{j=1}^n P_{ij} \log\left(\frac{P_{ij}}{Q_{ij}}\right). \quad (21)$$

For SPCC and SSIM, higher values denote better performance, while for RMSE and JS, lower values are preferable.

Results

Overview of STGENT

We present STGNET, a deep generative framework designed to overcome the gene panel limitation of imaging-based ST by accurately imputing the expression of unmeasured genes. The core challenge is to generate a complete, spatially coherent transcriptome from a limited initial measurement, thereby enabling more powerful downstream biological discovery.

As illustrated in Fig. 1, STGNET operates through a structured, two-stage pipeline. In the first stage, the model leverages a multi-stage GANs to learn the complex, high-dimensional distribution of gene expression from a reference scRNA-seq atlas. This phase is stabilized using the Wasserstein GAN with gradient penalty (WGAN-GP), ensuring robust learning of global transcriptomic features that are resilient to technical noise and dropout events common in single-cell data.

The second stage refines these initial imputations by explicitly modeling the spatial context of the tissue. Here, a multi-view GCN integrates two complementary graphs constructed from the ST data: a spatial graph, encoding physical proximity between cells/spots, and a feature graph, encoding transcriptional similarity. A key innovation is an integrated attention mechanism that dynamically fuses information from these two views, allowing the model to adaptively prioritize neighborhood context or cellular identity for each node. This ensures the final imputed expression matrix is not only transcriptionally plausible but also spatially consistent, faithfully capturing the tissue's structural organization. By synergistically combining global generative modeling with local spatial refinement, STGNET transforms sparse, targeted ST panels into comprehensive spatial transcriptomes, unlocking their full potential for analyses such as spatial domain identification, cell–cell communication inference, and trajectory analysis.

STGNET accurately imputes gene expression in spatial transcriptomics data

We rigorously evaluated the gene imputation performance of STGNET against seven state-of-the-art methods (i.e. Tangram, gimVI, SpaGE, stPlus, uniPort, SpatialScope, and stDiff) using a five-fold cross-validation framework. For each ST dataset, the gene panel was partitioned into five subsets. In each fold, one subset was held out as ground truth for validation, while the remaining four subsets were used as input to train the models. This procedure ensured a comprehensive assessment of each method's ability to predict unmeasured genes.

Performance was quantified using four complementary metrics: SPCC to assess monotonic relationships, SSIM to evaluate spatial pattern preservation, RMSE to measure numerical accuracy, and Jensen–Shannon divergence (JS) to compare expression distributions. We applied this evaluation to three different imaging-based ST datasets representing different biological backgrounds and technology

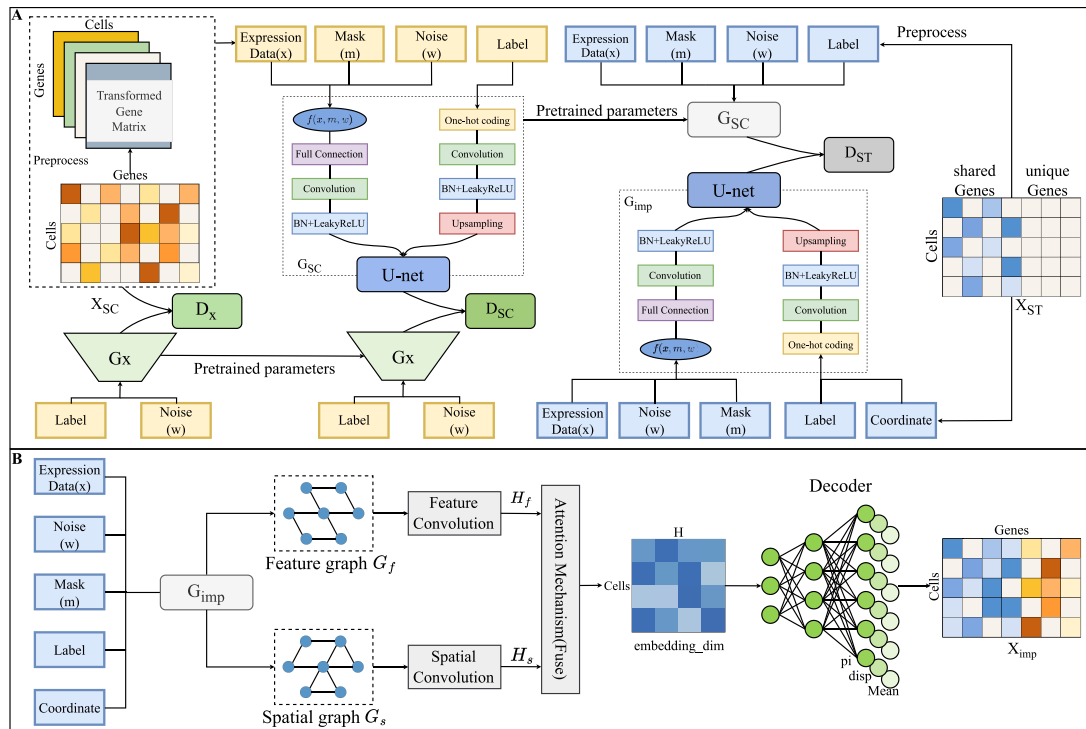


Figure 1 Overview of the STGNET framework for ST imputation. (A) Multi-stage GAN for initial imputation. A multi-stage GAN process learns the global transcriptome distribution from scRNA-seq data. Using shared genes between the scRNA-seq and ST datasets, it generates initial predictions for unmeasured genes in the ST data. (B) Spatial refinement with a multi-view GCN. A GCN refines the initial predictions using spatial context. Nodes represent cells/spots, with edges based on physical proximity and gene expression similarity. An attention mechanism fuses these views to produce spatially coherent imputations.

platforms: sequencing mouse embryonic using seqFISH technology, sequencing mouse primary motor cortex using MERFISH technology, and sequencing mouse osteosarcoma using MERFISH technology.

As shown in Fig. 2, STGNET demonstrated superior performance across all evaluation metrics and datasets. Notably, STGNET achieved the highest SPCC scores, with improvements ranging from 0.1145 to 0.1344 over the second-best performing methods, indicating stronger correlation between imputed and actual expression values. STGNET also excelled in preserving spatial expression patterns, as evidenced by superior SSIM scores, while simultaneously minimizing both numerical error and distributional divergence. The consistent outperformance across diverse datasets and multiple evaluation perspectives demonstrates STGNET's robust capability for accurate ST imputation.

STGNET preserves cellular topology and spatial domains

Accurate preservation of tissue topology is paramount for ST imputation, as the spatial organization of cells into distinct domains underpins fundamental biological processes. We therefore evaluated whether imputed data maintained the latent topological patterns present in the original tissue by comparing cluster assignments derived from imputed data against ground-truth annotations.

We first performed a detailed analysis on mouse primary motor cortex sequenced by MERFISH, which possesses authoritative cell type labels. Application of the Leiden algorithm to the data imputed by STGNET using the five-fold cross validation method and the full-transcriptome data revealed spatial domains that closely recapitulated

the original biological structure (Fig. 3A and B). Notably, clustering on STGNET-imputed data achieved higher concordance with the ground truth than clustering on the original, limited gene panel, indicating that STGNET not only preserves but can enhance the signal for spatial domain identification by augmenting the expression profiles.

To establish statistical robustness, we extended this validation across mouse embryonic sequenced by seqFISH, mouse gastrulation sequenced by seqFISH, primary visual cortex sequenced by ExSeq, somatosensory cortex sequenced by osmFISH, primary visual cortex sequenced by MERFISH and drosophila embryo sequenced by FISH six additional datasets using a five-fold cross-validation framework. The similarity between clusters from the imputed data and the ground-truth clusters was quantified using four established metrics (ARI, AMI, Homogeneity, and NMI). As summarized in Fig. 3C, STGNET consistently outperformed all benchmark methods, achieving the highest scores across all metrics and datasets. While methods like stDiff showed competitive performance on select datasets, their results were inconsistent. In contrast, STGNET reliably produced imputations that maintained the inherent topological structure of the tissue, underscoring its robustness and superiority for preserving the spatial integrity essential for downstream analyses. Comprehensive results are provided in Supplementary Tables S1 and S2.

For spatial clustering tasks, in order to verify the impact of gene imputation on performance. We validated on GraphST [35], DeepST [36], and Spaceflow [37] using raw and STGNET-imputed gene expression data in the Mouse primary motor cortex dataset. The experimental results show that imputation has improved performance in spatial clustering compared with the original data. The specific experimental results can be found in Supplementary Fig. S9.

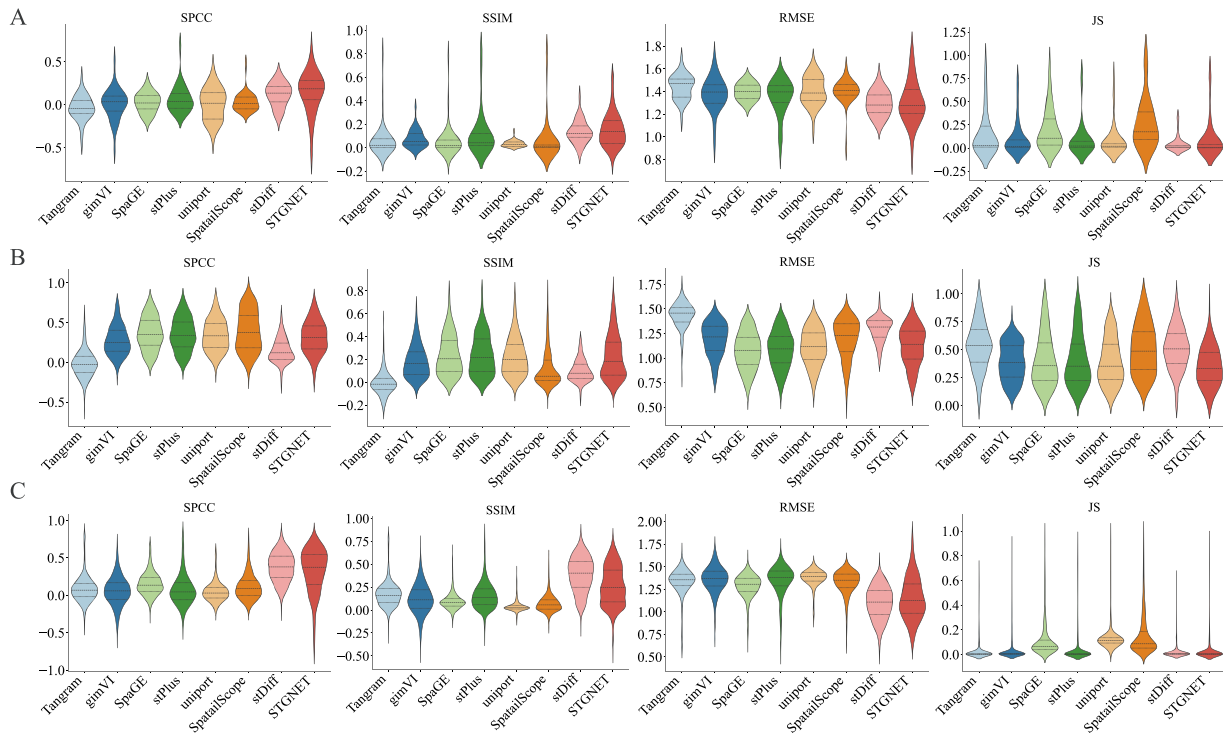


Figure 2 Benchmarking of gene imputation performance across (A) mouse embryonic, (B) mouse primary motor cortex, and (C) mouse osteosarcoma spatial transcriptomics datasets using SPCC, SSIM, RMSE, and JS demonstrates the superior performance of STGNET over seven state-of-the-art methods.

STGNET accurately reconstructs spatial expression patterns of key developmental genes

A critical challenge in ST imputation is the accurate reconstruction of biologically meaningful expression patterns for genes not included in the original panel. To evaluate this capability, we applied STGNET to a mouse gastrulation embryo dataset sequenced by seqFish, focusing on well-characterized marker genes with established spatial distributions across distinct tissue domains. Using a five-fold cross-validation framework, we masked these marker genes and assessed each method's ability to recover their characteristic spatial patterns.

We selected four key developmental markers with documented expression patterns: Sox2, a neural tube marker expressed in the brain and dorsal embryo regions [38]; Popdc2, a cardiomyocyte marker localized to the developing heart tube [39]; Foxa1, an endoderm marker expressed along the anterior–posterior axis of the gut tube [40]; and Foxf1, a mesoderm marker present in gut tube mesoderm, lateral plate mesoderm, and allantois [41].

As shown in Fig. 4B, STGNET successfully reconstructed the precise spatial distributions of all four marker genes, closely matching the patterns observed in the ground truth data. In contrast, other methods showed varying degrees of failure: stDiff, SpaGE, and stPlus generated diffuse or incorrect spatial patterns for most genes. While SpaGE and stPlus partially captured Sox2 expression, their reconstructions lacked the spatial precision achieved by STGNET. Notably, for Foxa1 and Popdc2, only STGNET accurately reconstructed the characteristic expression patterns, while all other methods failed. For Foxf1, although stDiff, SpaGE, and Tangram showed some spatial organization, their patterns lacked clear structural definition and contained regions inconsistent with the true biological distribution.

Quantitative analysis of Spearman correlation (Fig. 4C) confirmed STGNET's superior performance, with significantly higher SPCC values across all evaluated markers. Results for eight additional marker genes (Supplementary Fig. S4) further demonstrate STGNET's consistent ability to reconstruct biologically accurate spatial expression patterns, establishing its utility for discovering spatial gene distributions in developmental contexts.

STGNET reveals cancer progression trajectory and identifies novel markers in human breast cancer

Breast cancer progression involves a critical transition from ductal carcinoma *in situ* (DCIS) to invasive ductal carcinoma (IDC), a process that remains incompletely understood at the molecular level. To investigate this transition and identify potential drivers of invasion, we applied STGNET to a human breast cancer dataset generated by the Xenium platform, using the imputed whole transcriptome to reconstruct developmental trajectories and discover novel cancer-associated genes.

We used raw data for Louvain clustering and examined four relevant clustering metric values. The specific experimental results are ARI:0.3179, AMI: 0.5401, HOMO:0.5666, and NMI:0.5411. Under the same parameter settings, we examined the clustering metrics of the imputed data. The specific metric results are ARI: 0.4572, AMI: 0.7656, HOMO: 0.8896, and NMI: 0.7664. STGNET's imputation revealed substantially enhanced cellular heterogeneity compared with the original data. While Louvain clustering identified 11 distinct clusters in the original ST data (Fig. 5A), the imputed transcriptome resolved 20 fine-grained cellular communities (Fig. 5B). This enhanced resolution suggests that the complete transcriptome captured by

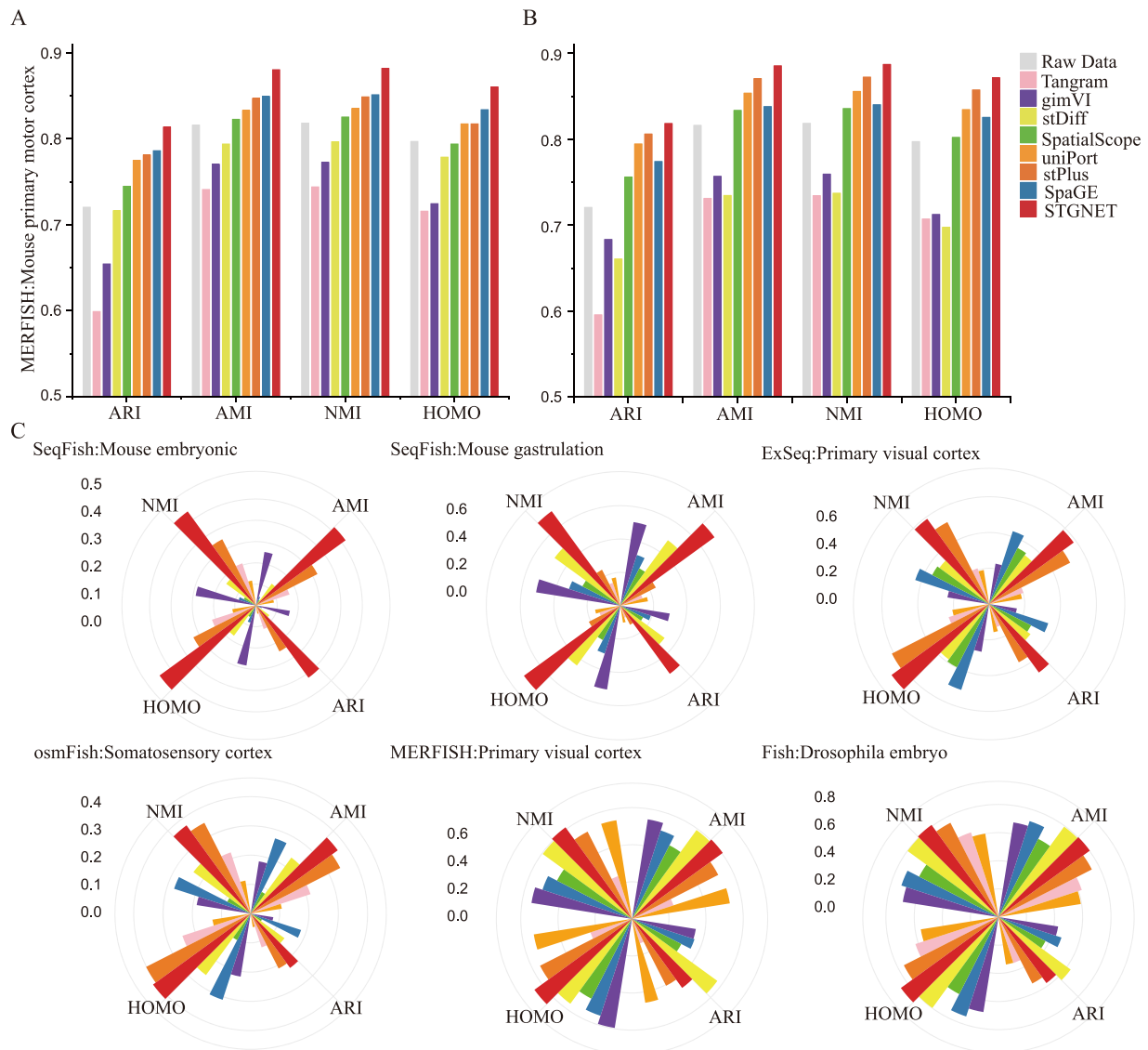


Figure 3 Comparison of clustering performance across multiple spatial transcriptomics datasets using ARI, AMI, NMI, and HOMO shows that STGNET achieves superior topological consistency between real ST data and imputed data compared with seven state-of-the-art methods.

STGNET uncovers previously obscured cellular states within the tumor microenvironment.

Trajectory inference using stLearn [42] on the imputed data revealed a more complex progression pathway from DCIS to IDC than was apparent in the original data. Whereas the original data suggested a direct transition from DCIS (Cluster 1) to Proliferative_Invasive_Tumor (Cluster 8), where Cluster 8 will develop into IDC, the imputed data identified an cellular state (Cluster 14) through which cells must pass during the DCIS-to-IDC transition (Fig. 5B). This intermediate state, which appears to be located in a region primarily labeled as **Unlabeled** in the original data, may represent a previously obscured cellular state that becomes statistically resolvable with enhanced whole-transcriptome context.

Leveraging the whole-transcriptome coverage enabled by STGNET, we identified several genes specifically associated with DCIS and proliferative invasive tumor clusters that are supported by existing cancer literature (Fig. 5C). These include CHI3L1 [43], known to be elevated in cancer tissues and implicated in carcinogenesis; DKK3,

frequently downregulated in malignant breast cancer cell lines [44]; EREG, enhanced in early breast lesions [45]; ITGB3, associated with tumor invasiveness and metastatic potential [46]; MUC16, overexpressed in breast cancer tissues [47]; and SCGB1D2, a highly specific breast cancer marker [48]. The identification of these established cancer genes validates STGNET's ability to recover biologically meaningful expression patterns, while also providing a platform for discovering novel candidates in the complete set of imputed genes.

STGNET enhanced differential expression analysis and marker gene detection

Due to the inherent sparsity and noise in ST data, many cell type-specific marker genes are difficult to detect in the original measurements, particularly those with low expression levels or layer-specific patterns. This sparsity limits the ability to resolve cellular subtypes and cortical laminar organization. Therefore, recovering masked biological signals and improving the sensitivity of cell-type marker detection

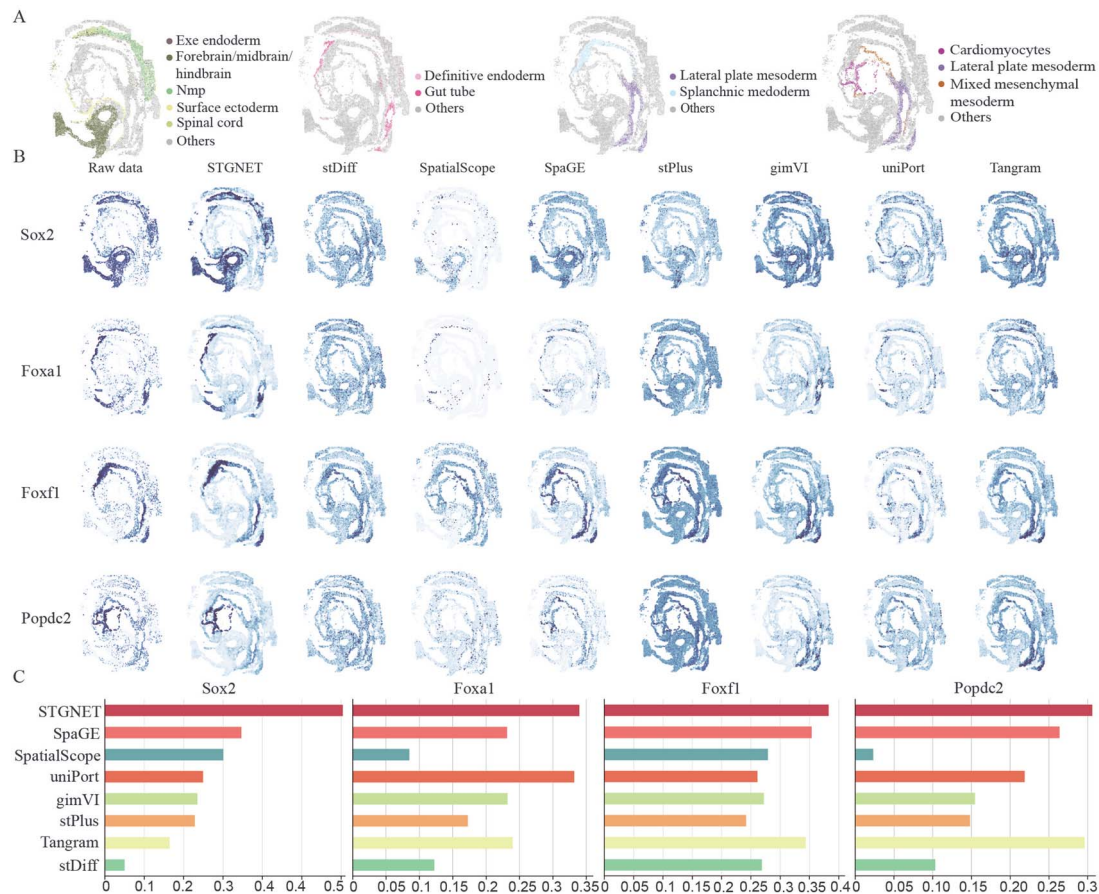


Figure 4 STGNET accurately reconstructs spatial expression patterns of key developmental markers in mouse embryogenesis. (A) Ground truth spatial distribution of cell types associated with four key developmental markers: Sox2 (neural tube), Foxa1 (endoderm), Foxf1 (mesoderm), and Popdc2 (cardiomyocytes). (B) Spatial expression patterns of the four marker genes. The first column shows the ground truth expression from the original ST data, while subsequent columns display imputations from different methods. STGNET accurately recapitulates the characteristic spatial distributions, including Sox2 in neural tissues, Foxa1 along the gut tube, Foxf1 in mesodermal regions, and Popdc2 in the developing heart. (C) Quantitative evaluation of imputation accuracy using Spearman correlation. STGNET achieves the highest correlation with ground truth expression patterns across all four marker genes, demonstrating its superior performance in reconstructing biologically meaningful spatial distributions.

remain key challenges in ST analysis. We demonstrated on the Mouse primary motor cortex dataset that it can more sensitively detect cell type specific markers.

STGNET enables the discovery of new marker genes that cannot be detected in the original gene expression matrix (Fig. 6A). Differential expression analysis identified *Clstn2* [49] as a novel layer-specific marker for L5 ET neurons (Fig. 6B), a finding supported by existing literature on cortical layer organization. Gene ontology enrichment analysis further demonstrated that the imputed data significantly enhanced the detection of neural function, tissue development, and synaptic activity pathways compared with the original data (Supplementary Figs S5 and S6).

These results collectively demonstrate that STGNET not only recovers known biology but also reveals novel insights into the complex signaling networks and molecular programs underlying motor cortex organization and function.

Computational performance and robustness analysis

To provide a comprehensive evaluation of STGNET's practical utility and architectural design, we conducted analyses across three dimensions: computational resource requirements, component contribution, and parameter sensitivity.

The time cost and memory usage experiments are conducted on an Ubuntu server with an Intel Sky Lake-E processor, GeForce RTX 2080 Ti GPU, and 256 GB of memory. We evaluated the runtime and peak memory consumption of STGNET against all seven benchmark methods on the Somatosensory Cortex dataset under a standardized five-fold cross-validation protocol. Runtime was recorded as the total wall-clock time for a complete training and imputation cycle per fold. Peak memory usage was monitored throughout the process. The results, detailed in Supplementary Table S3, reveal a clear spectrum of computational cost. Mapping-based method Tangram and the autoencoder-based (stPlus, gimVI) were computationally efficient, completing imputation within minutes using <1.1 GB of memory. In contrast, deep generative models requiring more complex optimization—including the diffusion-based methods (SpatialScope, stDiff) and our GAN-based STGNET—incurred significantly higher resource costs. STGNET required a mean runtime of ~1.72 h and 7 GB of peak memory per fold. This cost is attributed to the joint adversarial training of multiple generator–discriminator pairs and the subsequent message-passing operations of the GCN, which are intrinsic to achieving high-fidelity, spatially coherent imputations.

To rigorously evaluate the contribution of each core component within the STGNET architecture, we performed a systematic ablation study. We designed six distinct model variants, each removing a

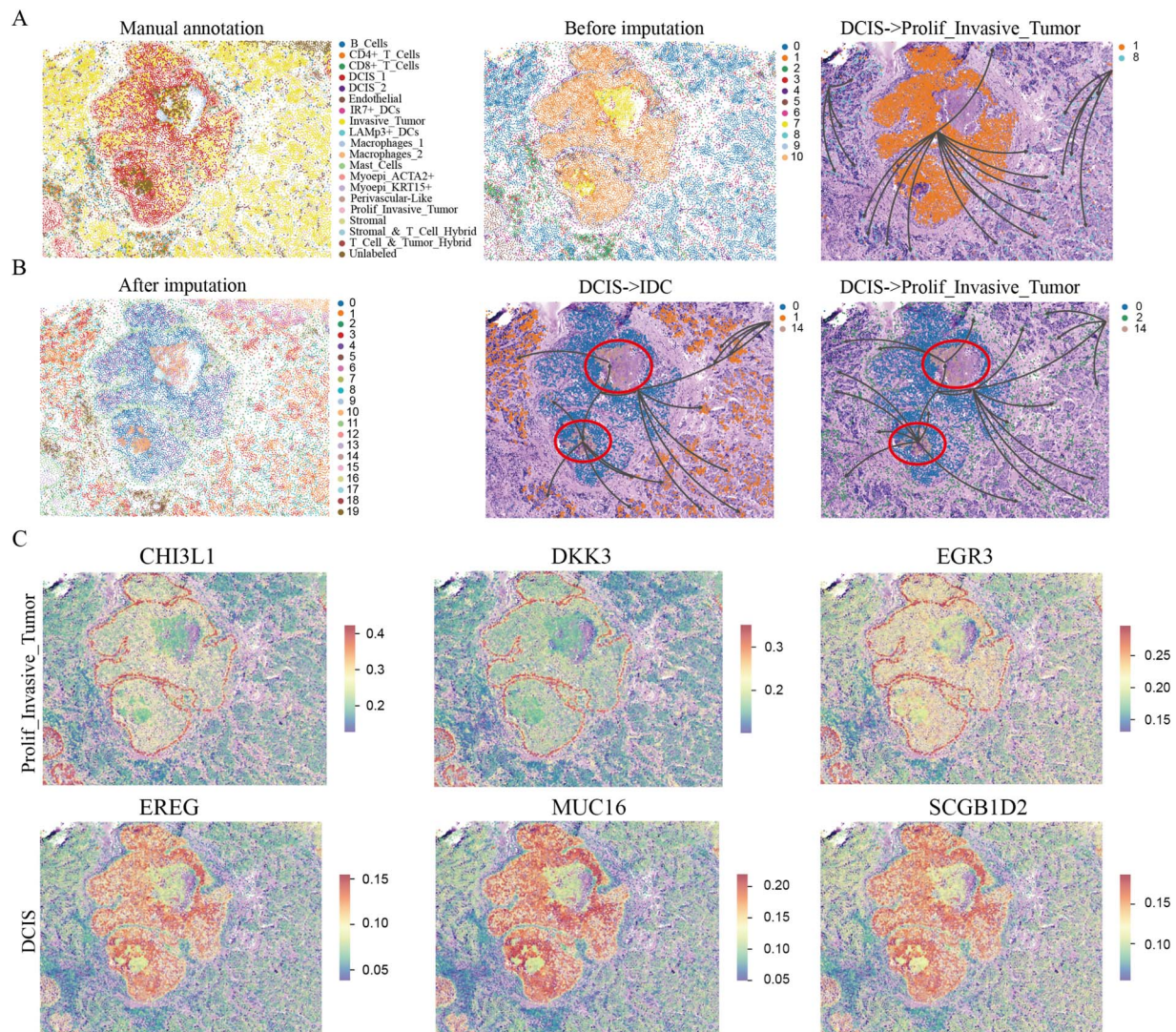


Figure 5 STGNET enables refined spatial trajectory inference and identifies cancer-associated genes in human breast cancer. (A) Manual cell type annotations from the Xenium breast cancer dataset. (Middle) Louvain clustering based on the raw dataset. (Right) The spatial trajectory inference from DCIS to Proliferative_Invasive_Tumor based on the raw dataset. (B) Enhanced cellular heterogeneity and progression trajectories revealed by STGNET. (Left) Louvain clustering applied to STGNET-imputed data reveals 20 distinct clusters. (Middle) Spatial trajectory inference using stLearn from DCIS-to-IDC, passing through cluster 14. (Right) Spatial trajectory inference using stLearn shows a more complex DCIS-to-Proliferative_Invasive_Tumor progression pathway through an intermediate cellular state (Cluster 14) that was not apparent in the original data. (C) Cancer-associated genes identified through whole-transcriptome imputation. (Top) Genes specifically associated with the proliferative invasive tumor (Prolif_Invasive_Tumor) cluster, including CHI3L1, DKK3, and EGR3. (Bottom) Genes associated with the DCIS cluster, including EREG, MUC16, and SCGB1D2. All identified genes have established roles in breast cancer pathogenesis, validating STGNET's ability to recover biologically meaningful expression patterns.

specific module or constraint from the full pipeline (see [Supplementary notes](#)). Each variant was evaluated on the Somatosensory Cortex dataset using our established five-fold cross-validation protocol. Performance was assessed using the full suite of gene-level (SPCC, SSIM, RMSE, and JS) and cluster-level (ARI, AMI, Homogeneity, and NMI) metrics. The results, detailed in [Supplementary Fig. S7](#) and [Table S4](#), provide quantitative validation of our architectural design. The most substantial performance degradation occurred when either the primary generative module (W/O G_{SC}) or the primary spatial module (W/O MG) was removed. This confirms that the two-stage paradigm—global distribution learning followed by local spatial refinement—is fundamental to STGNET's success. The ablation of individual graph views (W/O Spatial Graph, W/O Feature Graph) or auxiliary loss terms (W/O SR, W/O KL) resulted in consistent, though less severe, declines

across all metrics. This demonstrates that the multi-view graph integration and the composite loss function provide complementary information and constraints, collectively optimizing the imputed data for both transcriptional accuracy and spatial coherence.

To verify the robustness of the model, we conducted systematic experiments on the imputed results obtained using the five-fold cross-validation method for the hyperparameters on the Somatosensory Cortex dataset. The experimental results are shown in [Supplementary Fig. 8](#) and [Tables S5](#) and [S6](#). The experimental results indicate that STGNET requires an appropriate λ to maintain the stability of GAN training. At the same time, a suitable α is needed to ensure a balance between adversarial loss and reconstruction loss during the training process. When $r = 4$ and $k = 500$, the model reaches its optimum. As these two values increase, redundant

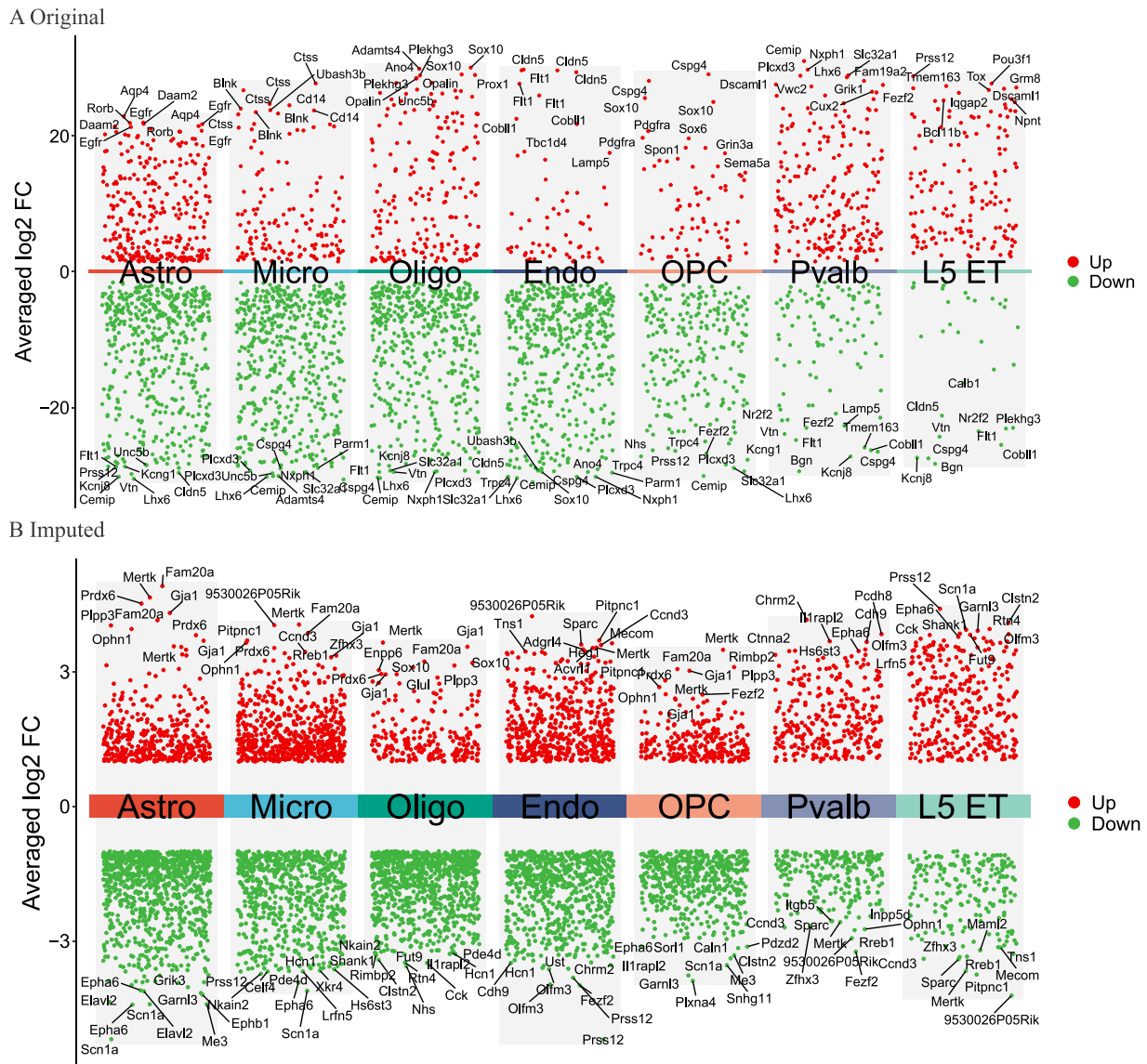


Figure 6 Differential gene expression analysis of original and imputed spatial transcriptomics data on mouse primary motor cortex. (A) The differential expression analysis results of the raw data show limited DEGs. (B) Differential expression analysis identifies *Cln2* as a novel layer-specific marker for L5 ET neurons in the imputed data, demonstrating STGNET's enhanced sensitivity for detecting cell-type-specific genes.

information may be introduced. Typically, four neighboring cells are selected to construct the adjacency graph. The appropriate r is determined based on the number of k -nearest neighbors to construct a gene expression similarity adjacency matrix with consistent numbers. At the same time, we are also concerned about the hyperparameters of imputation optimization objectives in multi-view graph neural network. Experiments were conducted, and the results showed that various parameter combinations had little effect on gene similarity indicators. These experiments effectively demonstrate the robustness of STGNET.

Conclusion

In this study, we present STGNET, a novel deep learning framework that integrates GANs and GCNs to address the fundamental limitation of gene panel size in imaging-based ST. By leveraging a multi-stage GAN architecture, STGNET first learns the continuous distribution of

gene expression from single-cell RNA sequencing references, generating transcriptomically plausible data. The model further refines these imputations through a spatially-aware graph neural network that explicitly incorporates both physical proximity and transcriptional similarity, ensuring the final predictions maintain biological coherence within the tissue architecture.

Our comprehensive evaluation across nine diverse datasets demonstrates that STGNET consistently outperforms existing state-of-the-art methods in both gene-level accuracy and structural preservation. Quantitative assessments using multiple clustering and similarity metrics confirm that STGNET produces imputed data that closely matches ground truth measurements while maintaining the topological relationships essential for spatial biology. More importantly, the practical utility of STGNET is evidenced through multiple biological applications: it successfully reconstructed key developmental gene expression patterns in mouse embryogenesis, and revealed novel transitional states in breast cancer progression.

The ability of STGNET to extend limited gene panels to comprehensive transcriptome coverage while preserving spatial context represents a significant advance in ST analysis. By enabling more complete characterization of cellular heterogeneity, developmental processes, and disease mechanisms, STGNET provides researchers with a powerful tool to extract deeper biological insights from existing ST datasets. As the field continues to evolve, we anticipate that computational approaches like STGNET will play an increasingly vital role in maximizing the information yield from spatial genomics technologies and advancing our understanding of tissue organization and function in both health and disease.

Key Points

- This study proposed STGNET, a novel deep learning framework that uniquely integrates multi-stage generative adversarial networks with a spatially-aware graph convolutional network to address the gene panel limitation in imaging-based spatial transcriptomics (ST).
- STGNET outperforms seven state-of-the-art methods across nine diverse datasets, demonstrating superior accuracy in gene expression imputation and exceptional preservation of cellular topological structures.
- STGNET provides a robust computational solution to extend the effective coverage of targeted ST assays, transforming sparse, panel-based data into comprehensive ST for deeper, and more reliable biological investigation.
- STGNET enables new biological discoveries, including reconstructing key developmental gene patterns, identifying a novel transitional cell state in breast cancer progression, and uncovering extensive cell–cell communication networks previously obscured by limited gene panels.

Author contributions

T.W., B.X., P.Z., and B.W. conceived the experiments. B.W., J.C., and T.W. conducted the experiments. T.W., J.C., J.H., J.P., X.S., and Z.W. analyzed the results. B.W., H.S., T.W., and Z.W. wrote and reviewed the manuscript. T.W. supervised the research and provided funding support. All authors contributed to the manuscript and approved the final version.

Supplementary material

Supplementary material is available at *Briefings in Bioinformatics* online.

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Conflicts of interest

None declared.

Data availability

The datasets and model source code used for training and testing are available at <https://github.com/wuyuanwuhui/STGNET>.

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