Multi-modal Topology-embedded Graph Learning for Spatially Resolved Genes Prediction from Pathology Images with Prior Gene Similarity Information

Supplementary Material

6. Description of the comparing methods

For the purpose of evaluating the effectiveness of our M2TGLGO, we compare it against the following seven spatial resolved gene prediction methods:

1) Hist2ST[42]: A ST based gene prediction method that utilizes histology jointly with Transformer and Graph Neural Networks.

2) HisToGene[27]: A deep learning model for gene expression prediction from histology images by leveraging Transformer.

3) THItoGene[18]: A hybrid neural network that utilizes dynamic convolutional and capsule networks to adaptively sense potential molecular signals in histological images.

4) ST-Net[14]: A method that links gene expression with visual features in cell morphology via deep neural networks.

5) BLEEP[39]: A bi-modal embedding framework capable of generating spatial resolved gene expression profiles of pathological images.

6) TRIPLEX[5]: A novel spatial resolved gene prediction method by integrating multi-resolution features.

7) TG-GATEs[17]: Leveraging complementary information from gene expression profiles to guide slide representation learning.

In addition, we also compare our method with six pathological image representation or multi-modal integrative analysis methods, including:

1) IGI-DL[11]: A deep learning system that can augment tumor micro-environment information for pathological image representation.

2) SI-MIL[19]: Employs a deep multi-instance learning framework to guide an interpretable branch grounded on handcrafted pathological features.

3) TMEGL[32]: A tumor micro-environment interactions guided graph learning for the representation of whole-slide histopathology images.

4) MGNN[10]: A multi-modal graph neural network for the combinations of different types of features.

5) GECMC[38]: A contrastive multi-modal learning framework that can explore complementary information from diverse modalities.

6) TSIEN[34]: A multi-view multi-task learning framework that can minimize task-irrelevant information while maximizing task-relevant information through the principles of information bottleneck theory.

7. Effects of similarity nodes number for gene prediction.

In section 3.5 of the main text, we set the number of most similar nodes as 10 to dynamic adjust the parameter θ for constructing the graph G_g. Here, we tune the similarity nodes number from 6 to 12 with interval 2, and reports their gene prediction results in Table 11 and Table 12. As can be seen from these tables, our method can achieve superior gene prediction performance when the number of most similar nodes is set as 10. On the other hand, we do not find significant difference with diverse number of most similar nodes, which suggests that our method is relatively robust to that parameter.

8. Example of Localized Directed Acyclic Graph (DAG) within Gene Ontology (GO)

The Gene Ontology (GO) is a standardized framework for describing gene functions and their products, extensively used in gene function analysis. GO consists three primary categories: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). The terms are structured in a Directed Acyclic Graph (DAG), where each node represents a GO term, and each edge indicates hierarchical relationships (*e.g.,* "is-a" signifies "a type of," and "part-of" refers to "a part of"). Unlike a tree structure, the DAG allows each term to have multiple parent nodes, thereby capturing the multidimensional nature of biological functions.

In Fig. 7, we show the hierarchical structure of BP branch as follows. At the top level, the term "biological process (GO:0008150)," encompasses all fundamental biological activities. In the second level, the term "biological regulation (GO:0065007)," represents processes that modulate measurable attributes of biological activities. In the third level, "regulation of biological process (GO:0050789)," focuses on maintaining proper balance in biological processes. This is followed by terms such as "signaling (GO:0023052)," which describes the transmission of biochemical signals, and "cell-cell signaling (GO:0007267)," referring to communication between distinct cells. All these GO terms construct a localized Directed Acyclic Graph (DAG).

Table 4. Effects of similarity nodes number for gene predictions by the measurement of PCC.

Gene	DLPFC		ccRCC		BC	
Neighbors	PCC(HVG)	PCC(HEG)	PCC(HVG)	PCC(HEG)	PCC(HVG)	PCC(HEG)
6	0.253	0.204	0.266	0.235	0.250	0.244
8	0.266	0.201	0.273	0.246	0.306	0.263
10	**0.291**	**0.217**	**0.296**	**0.256**	**0.308**	**0.269**
12	0.267	0.213	0.278	0.250	0.293	0.220

Table 5. Effects of similarity nodes number for gene predictions by the measurement of MSE.

Gene	DLPFC		ccRCC		BC	
Neighbors	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)
6	0.141	0.111	0.203	0.156	0.161	0.147
8	0.132	0.104	0.198	0.145	0.173	0.151
10	**0.130**	**0.101**	**0.184**	**0.141**	**0.157**	**0.137**
12	0.135	0.102	0.186	0.169	0.165	0.142

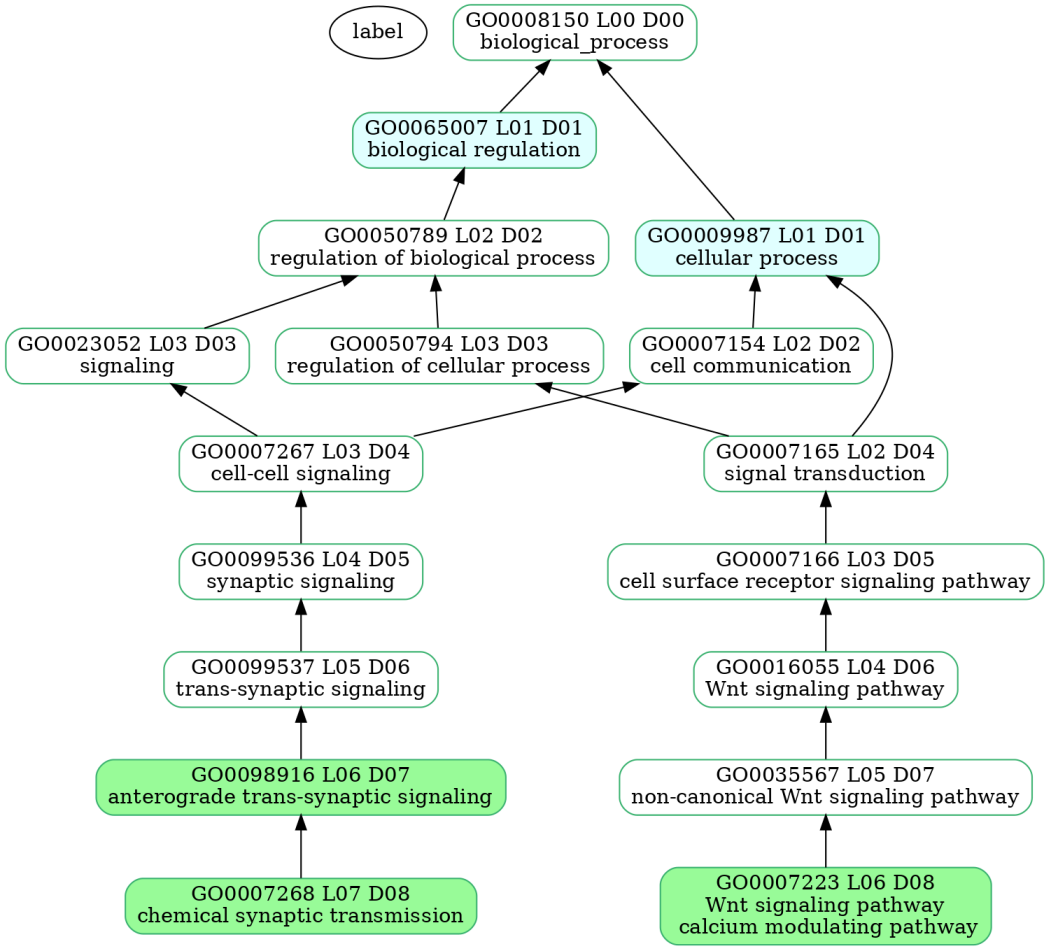

Figure 7. Hierarchical structure of gene ontology terms in the biological process(BP) branch.

Table 6. Descriptions of 14 morphological features of nuclei in our study.

Feature	**Description**
Area	Number of pixels in the nuclei.
Perimeter	The total length of the nuclei boundary.
Eccentricity	Eccentricity of the nuclei.
MajorAxisLength	Length of the major axis of the nuclei.
MinorAxisLength	Length of the minor axis of the nuclei.
Solidity	Ratio of pixels in the region to pixels of the convex hull image.
Extent	Ratio of pixels in the region to pixels in the bounding box.
Orientation	Angle between the 0th axis (rows) and the major axis of the nuclei.
Circularity	Circularity of the nuclei.
AspectRatio	Ratio of the major axis length to minor axis length.
ConvexArea	Area of the smallest convex polygon enclosing the nuclei.
Convexity	Ratio of the convex hull perimeter to the actual perimeter.
SkeletonLength	Length of the region's skeleton after thinning.
ConvexPerimeter	Perimeter of the nuclei's convex hull.

Table 7. Overview of Spot and Gene Counts in the DLPFC, ccRCC, and BC Datasets

DLPFC			**ccRCC**			**BC**		
Dataset ID	Spot Count	Gene Count	Dataset ID	Spot Count	Gene Count	Dataset ID	Spot Count	Gene Count
151507	4226	33538	GSM4284316	666	17138	A1	346	15045
151508	4384	33538	GSM4284317	646	17344	A2	325	15526
151509	4789	33538	GSM4284318	638	17883	A3	359	15517
151510	4634	33538	GSM4284319	590	16959	A4	343	15583
151669	3661	33538	GSM4284320	521	17689	A5	332	15638
151670	3498	33538	GSM4284321	521	17399	A6	360	15645
151671	4110	33538	GSM4284322	1145	17823	B1	295	15109
151672	4015	33538	GSM4284323	1071	19314	B2	270	15290
151673	3639	33538	GSM4284324	1182	17976	B3	298	15215
151674	3673	33538	GSM4284325	608	15383	B4	283	15289
151675	3592	33538	GSM4284326	621	16642	B5	289	15273
151676	3460	33538	GSM4284327	462	17047	B6	277	15387

Table 8. Average Mean Squared Error (MSE) of predicted expression for the top 50 highly expressed genes (HEG) and top 50 highly variable genes (HVG) compared to ground truth expressions. The '*' symbol indicates that our M2TGLGO is significantly better than the comparing methods.

	DLPFC		**ccRCC**		**BC**	
	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)
Hist2ST[42]	0.234 ± 0.0299 *	0.296 ± 0.0053 *	0.324 ± 0.1284 *	0.312 ± 0.1103 *	0.271 ± 0.0873 *	0.237 ± 0.0136 *
HisToGene[27]	0.207 ± 0.0701 *	0.249 ± 0.1182 *	0.291 ± 0.0520 *	0.242 ± 0.1405 *	0.353 ± 0.1095 *	0.313 ± 0.0012 *
THitoGene[18]	0.341 ± 0.0123 *	0.311 ± 0.0499 *	0.287 ± 0.0795 *	0.265 ± 0.0043 *	0.301 ± 0.0694 *	0.299 ± 0.0071 *
ST-Net[14]	0.292 ± 0.1344 *	0.307 ± 0.0341 *	0.293 ± 0.0385 *	0.232 ± 0.0154 *	0.258 ± 0.0128 *	0.251 ± 0.0108 *
TRIPLEX[5]	0.225 ± 0.0556 *	0.219 ± 0.0536 *	0.258 ± 0.0875 *	0.202 ± 0.0751 *	0.226 ± 0.0959 *	0.212 ± 0.1235 *
TG-GATEs[17]	0.214 ± 0.0307 *	0.209 ± 0.0416 *	0.227 ± 0.0265 *	0.259 ± 0.0394 *	0.262 ± 0.0131 *	0.284 ± 0.0762 *
BLEEP[39]	0.181 ± 0.0759 *	0.134 ± 0.0679 *	0.238 ± 0.0788 *	0.214 ± 0.1003 *	0.206 ± 0.0430 *	0.168 ± 0.0343 *
M2TGLGO	**0.130 ± 0.0185**	**0.101 ± 0.0217**	**0.184 ± 0.0274**	**0.141 ± 0.0284**	**0.157 ± 0.0313**	**0.137 ± 0.0172**

Table 9. Ablation studies for different Modules in our M2TGLGO by the measurement of MSE.

MMGE		**SONRM**	**GO Prior**	**DLPFC**		**ccRCC**		**BC**	
Within-Modal	Inter-Modal		**Knowledge**	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)
	✓	✓	✓	0.212	0.178	0.292	0.248	0.254	0.316
✓		✓	✓	0.169	0.194	0.191	0.219	0.231	0.180
✓	✓		✓	0.227	0.200	0.216	0.186	0.213	0.241
✓	✓	✓		0.229	0.289	0.266	0.252	0.206	0.274
✓	✓	✓	✓	**0.130**	**0.101**	**0.184**	**0.141**	**0.157**	**0.137**

Table 10. Comparisons of our method with its competitors relying on partial modality data by the measurement of PCC.

Modalities	DLPFC		ccRCC		BC	
	PCC(HVG)	PCC(HEG)	PCC(HVG)	PCC(HEG)	PCC(HVG)	PCC(HEG)
D, M, T	**0.291**	**0.217**	**0.296**	**0.256**	**0.308**	**0.269**
D, M	0.232	0.156	0.247	0.211	0.245	0.188
D, T	0.194	0.167	0.178	0.224	0.148	0.101
M	0.071	0.057	0.159	0.154	0.122	0.119
T	0.082	0.039	0.103	0.015	0.081	0.006

Table 11. Comparisons of our method with its competitors relying on partial modality by the measurement of MSE.

Modalities	DLPFC		ccRCC		BC	
	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)
D, M, T	**0.130**	**0.101**	**0.184**	**0.141**	**0.157**	**0.137**
D, M	0.190	0.201	0.239	0.199	0.236	0.228
D, T	0.187	0.166	0.243	0.212	0.217	0.205
M	0.289	0.290	0.251	0.340	0.345	0.230
T	0.374	0.310	0.233	0.330	0.351	0.237

Table 12. Performance comparison with various Quantile values by the measurements of MSE.

Quantile	DLPFC		ccRCC		BC	
	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)	MSE(HVG)	MSE(HEG)
3	0.133	0.112	0.181	0.137	0.155	0.140
4	**0.130**	**0.101**	**0.184**	**0.141**	**0.157**	**0.137**
5	0.128	0.110	0.193	0.142	0.146	0.132
6	0.136	0.107	0.179	0.140	0.151	0.134

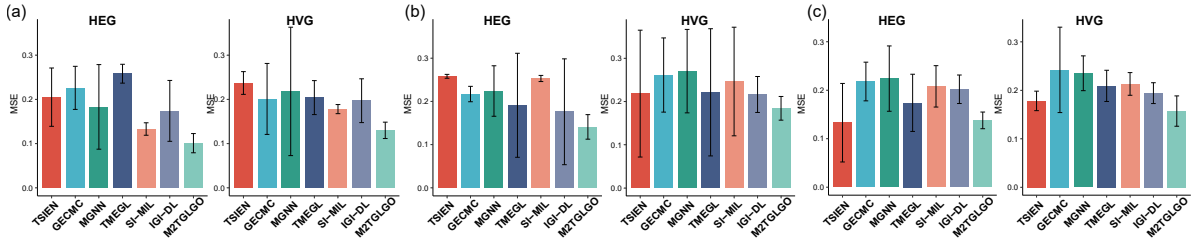

Figure 8. Comparison of M2TGLGO with other image representation methods on (a) DLPFC, (b) ccRCC and (c) BC datasets by the measurement of average MSE.

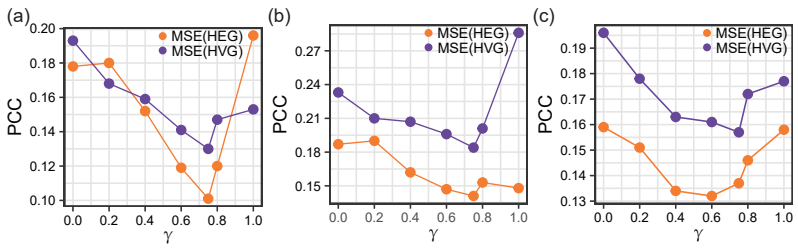

Figure 9. Performance of the model with varying values of hyperparameter γ on (a) DLPFC, (b) ccRCC, and (c) BC datasets by the measurement of MSE.