**Reviewer Report**

**Title: Deciphering spatial domains from spatially resolved transcriptomics with Siamese Graph Autoencoder**

**Version: Original Submission** **Date:** 12/12/2023

**Reviewer name: Jia Song**

**Reviewer Comments to Author:**

The paper entitled "Deciphering spatial domains from spatially resolved transcriptomics with Siamese Graph Autoencoder" by Lei Cao, et al. explores an innovative framework called SGAE for spatial domain identification in spatial transcriptomics (ST) data analysis. This framework addresses the limitations of existing methods by incorporating the power of Siamese Graph Autoencoder (SGAE) to improve representation discrimination. The article also highlights the potential of SGAE in enhancing the accuracy of identifying 3D Drosophila embryonic structures. Nevertheless, several significant concerns remain regarding the author's stated conclusion.1ï¼‰ The author mentioned "GNN-based methods suffer from representation collapse, wherein all spatial spots are projected onto a singular representation." and "SGAE mitigates the information correlation at the both sample and feature level, thus improving the representation discrimination." However, the author did not provide enough evidence to demonstrate the ability of SGAE in solving representation collapse, nor did they prove that the GNN approach indeed leads to such problems. More analysis evidence required to support the role of SGAE in representation collapse. Additionally, it would be helpful to understand the impact of representation collapse on downstream bioinformatic analysis.2ï¼‰ Did the Siamese architecture make a difference on the performance of spatial domain identification? Further ablation experiments are needed to demonstrate the effectiveness of each module in the SGAE architecture.3ï¼‰ The author only analyzed the trajectory of one mouse brain sample. This is still far from proving that the embeddings generated by SGAE can exhibit better performance in downstream applications. Does the result of trajectory inference correspond to the actual biological development process?4ï¼‰ Did author adapt proper parameter on the other candidate models? Or is there any different on the preprocess of SGAE and other candidate method? Some of the candidate methods showed significantly poorer results compared to what was reported in corresponding paper, For example the Fig2 A.5ï¼‰ The author mainly used K-means to generate a pseudo-label when pre-clustering. While Louvain or Leiden is used to preform clustering on cell embeddings. Is there any preference of SGAE on choose the pre-cluster method and final cluster method?6ï¼‰ It would be helpful to include a comprehensive hyperparameters table in order to elucidate the relative impact of each parameter on the model's performance. This table should also provide insights into the potential consequences of adjusting these parameters to higher or lower values.7ï¼‰ The author declaimed little batch effect detected on 3D Drosophila datasets. Is there any evidence? How would SGAE performance on multi-slice datasets with high batch effect.

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