



Benchmarking of single-cell trajectory inference algorithms

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Introduction

- **Trajectory inference** orders single-cell RNA-seq profiles along a reconstructed developmental timeline to model dynamic biological processes.
- **Systematic benchmarking** is essential given the proliferation of >70 TI methods with varied assumptions and outputs.
- This study evaluates **7 popular TI algorithms** using **4 complementary metrics** (HIM, F1_branches, Correlation, FeatureImp_wcor) across **4 diverse datasets**.

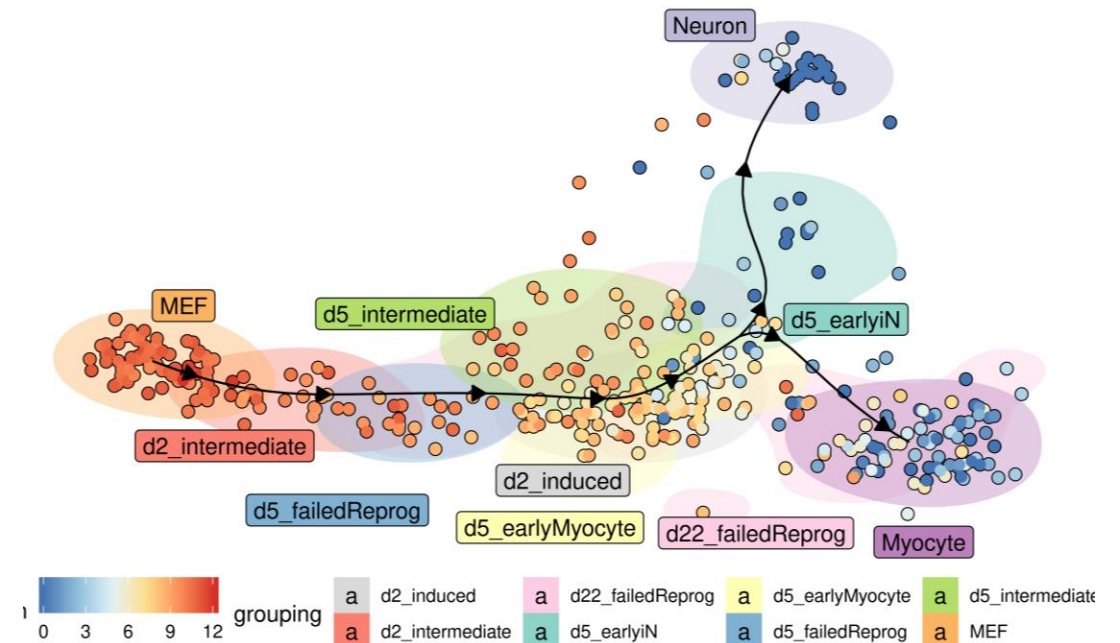


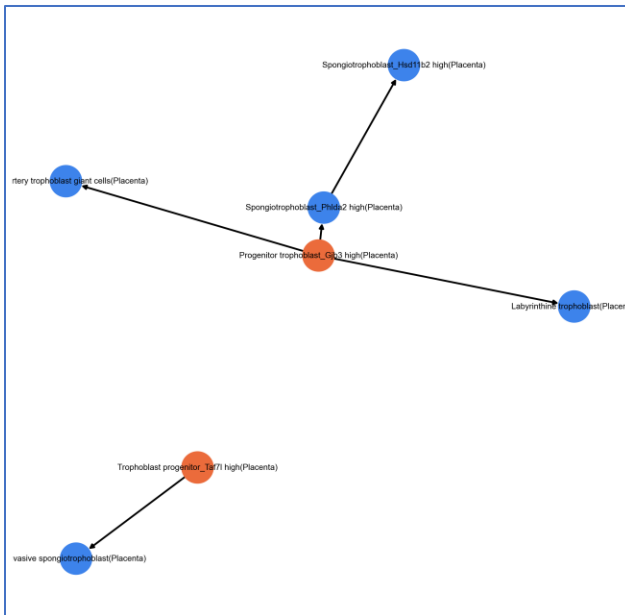
Image source: <https://dynverse.org/dyno/>

Datasets

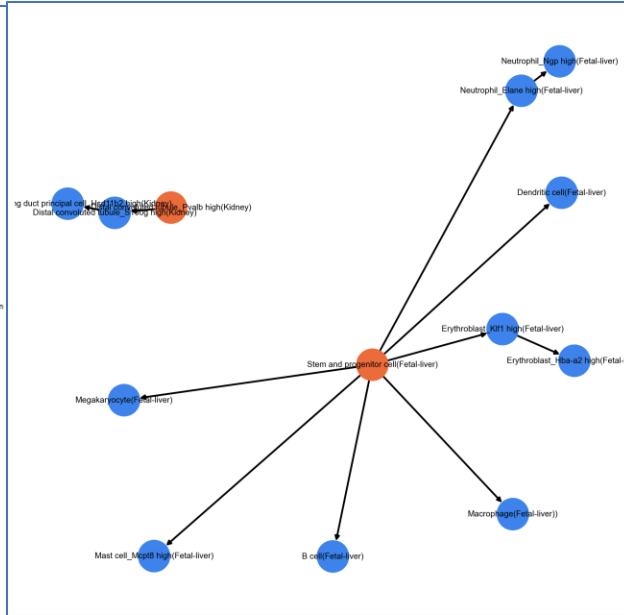
Dataset	Organism	No. of Cells	No. of Genes	Topology	Tissue	Reference
Placenta Trophoblast differentiation	Mouse	1740	2588	Disconnected	Placenta	[9]
Mouse cell atlas	Mouse	3211	2218	Disconnected	General	[9]
Oligodendrocyte differentiation	Mouse	4930	3534	Multifurcating	Brain	[10]
Planaria parenchyme differentiation	Planaria	1986	4210	Multifurcating	Parenchyme	[11]

Datasets source: Kushagra Pandey. (2022). Simulated and Real datasets used for benchmarking MARGARET (1.0.0) [Data set]. Zenodo. <https://doi.org/10.5281/zenodo.5850115>

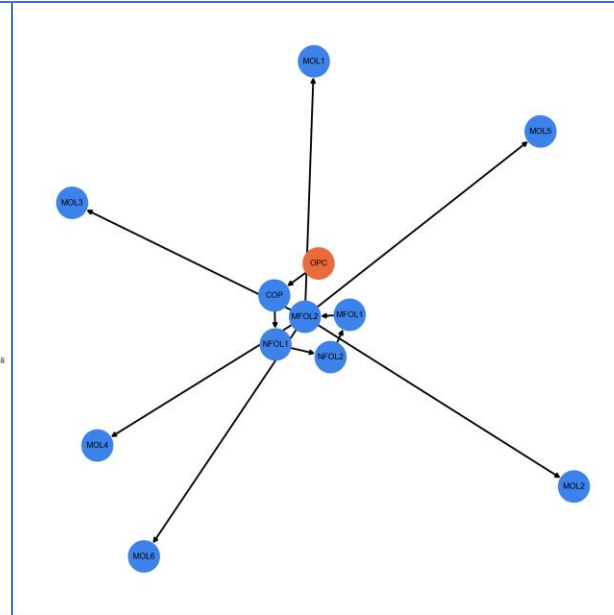
Datasets



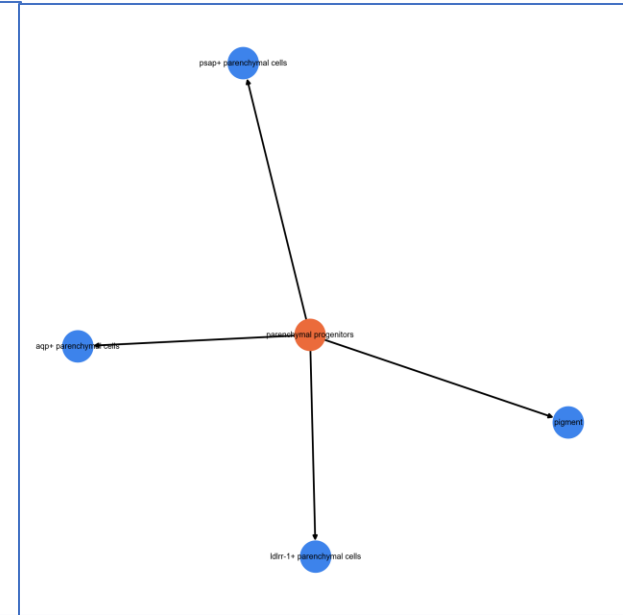
Placenta Trophoblast differentiation



Mouse cell atlas



Oligodendrocyte differentiation



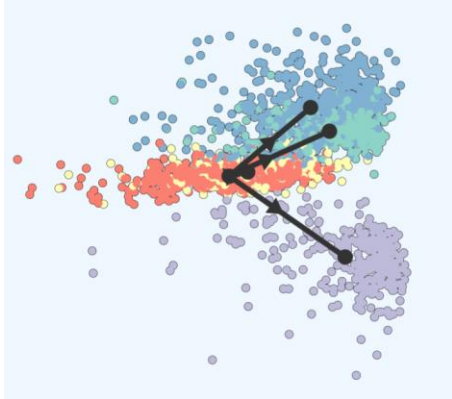
Planaria Parenchyme differentiation

Trajectory Inference Methods

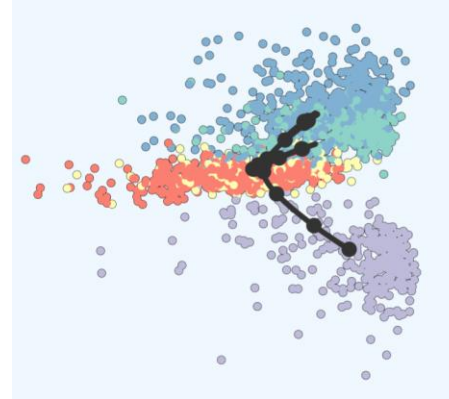
Method	Algorithm	Dim. Reduction	Topology	Platform
VIA	Lazy teleporting random walks	None	Disconnected	Python
MARGARET	Deep unsupervised metric learning	Metric learning based embedding	Disconnected	Python
Palantir	Markov Chain	Diffusion Map	Tree	Python
PAGA	Graph abstraction on KNN graph	PAGA-initialized manifold learning	Disconnected	Python
Slingshot	MST + Principal Curves	Any	Tree	R
DPT	Diffusion distances	Diffusion Map	Bifurcation	R
SCORPIUS	Correlation distances	Multi-dimensional scaling	Linear	R

Trajectory Inference Methods

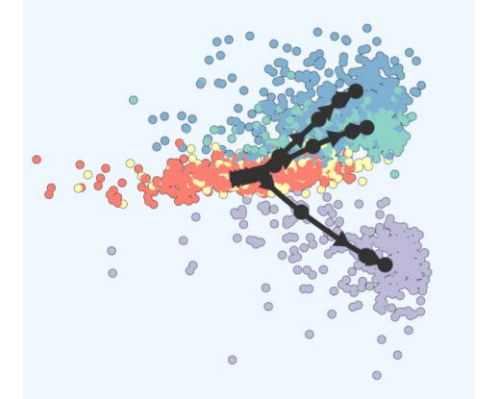
Dataset: Planaria
Parenchyma differentiation



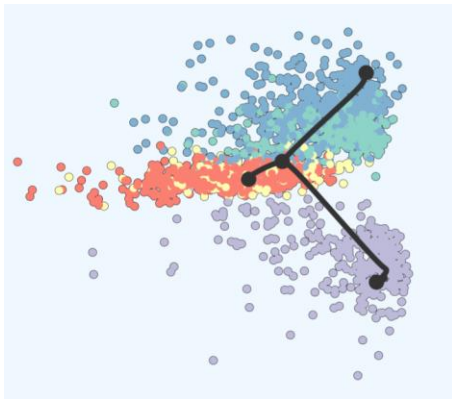
Ground Truth



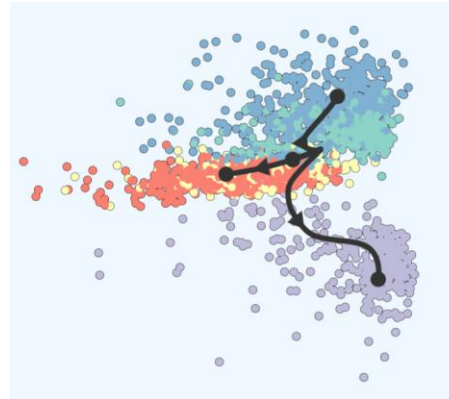
VIA



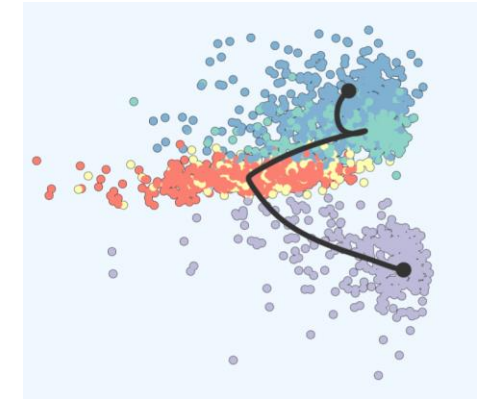
MARGARET



DPT



Slingshot



SCORPIUS

Metrics

HIM Similarity

- Measures **Topological Similarity**
- Combines Hamming distance and Ipsen-Mikhailov distance.
- Formula: $HIM = 1 - \sqrt{0.5 \cdot (H^2 + I^2)}$
- Range: 0 to 1 (higher the better)
- Significance: Verifies if overall branching structure matches the reference.

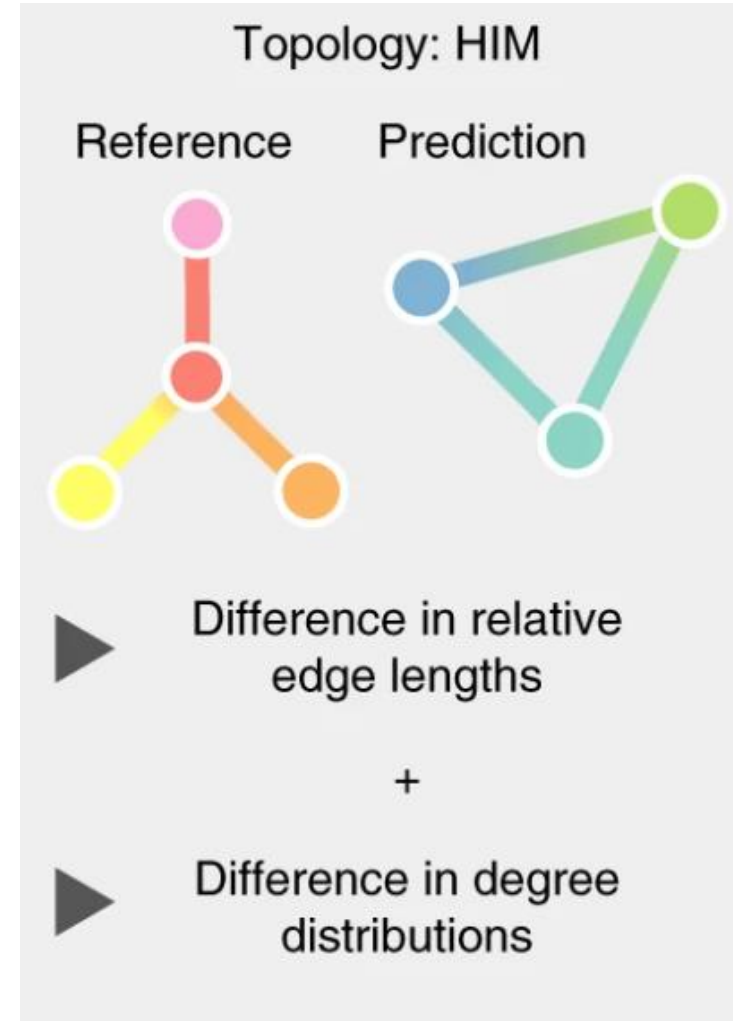


Image source: <https://www.nature.com/articles/s41587-019-0071-9/figures/1>

Metrics

F1_branches

- Accuracy of **cell-to-branch** assignments
- Maps cells to closest branches, then calculates F1-score.
- Formula: $F1 \text{ score} = HM(\text{Precision}, \text{Recall})$
- Range: 0 to 1 (higher the better)
- Significance: Confirms cells follow correct developmental lineages.

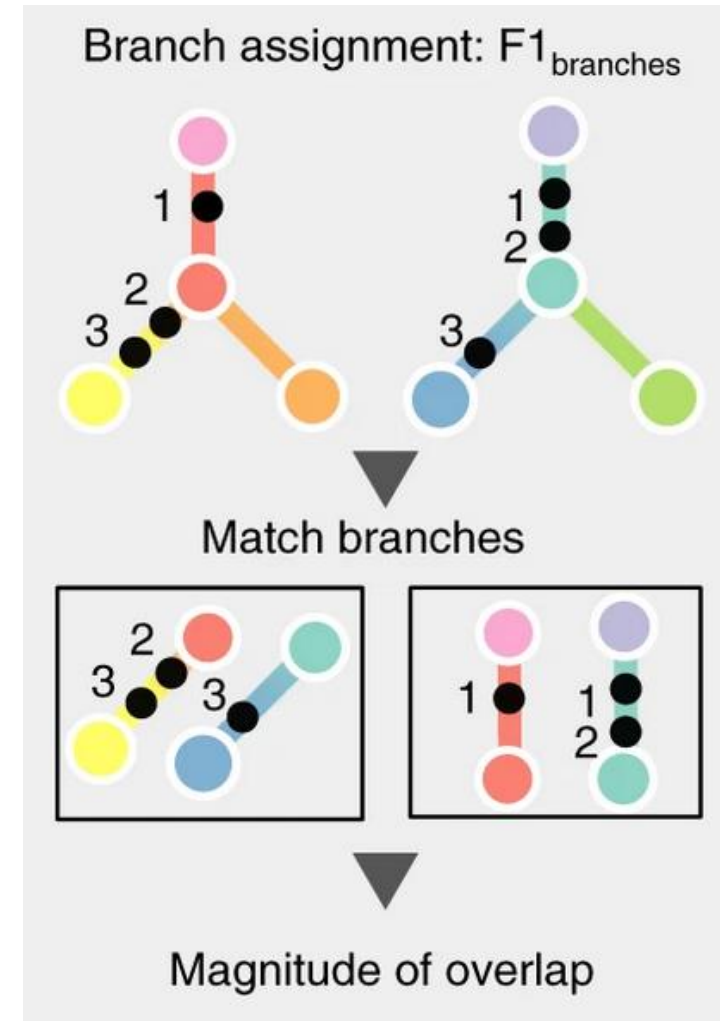


Image source: <https://www.nature.com/articles/s41587-019-0071-9/figures/1>

Metrics

Correlation

- Preservation of cell ordering
- Calculates Spearman correlation between **geodesic distances** in the two trajectories.
- Range: 0 to 1 (higher the better)
- Significance: Verifies relative timing of differentiation events is correct.

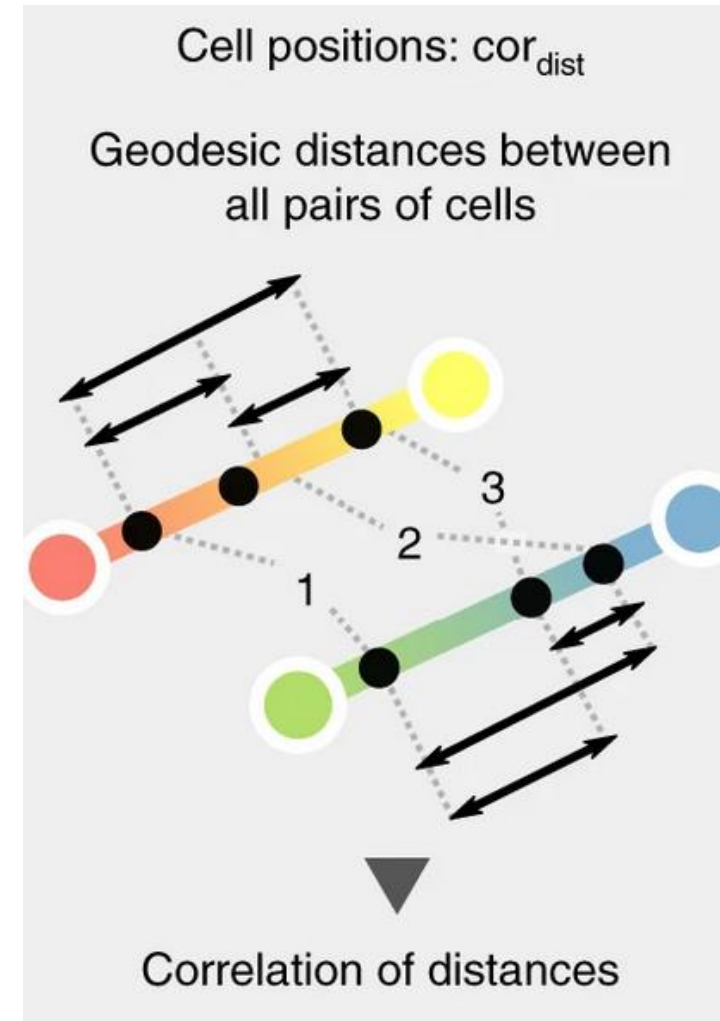


Image source: <https://www.nature.com/articles/s41587-019-0071-9/figures/1>

Metrics

Featureimp_wcor

- Agreement on biologically important genes
- Calculates weighted correlation between important genes of the two trajectories.
- Range: 0 to 1 (higher the better)
- Significance: Confirms **biological relevance** of the trajectory.

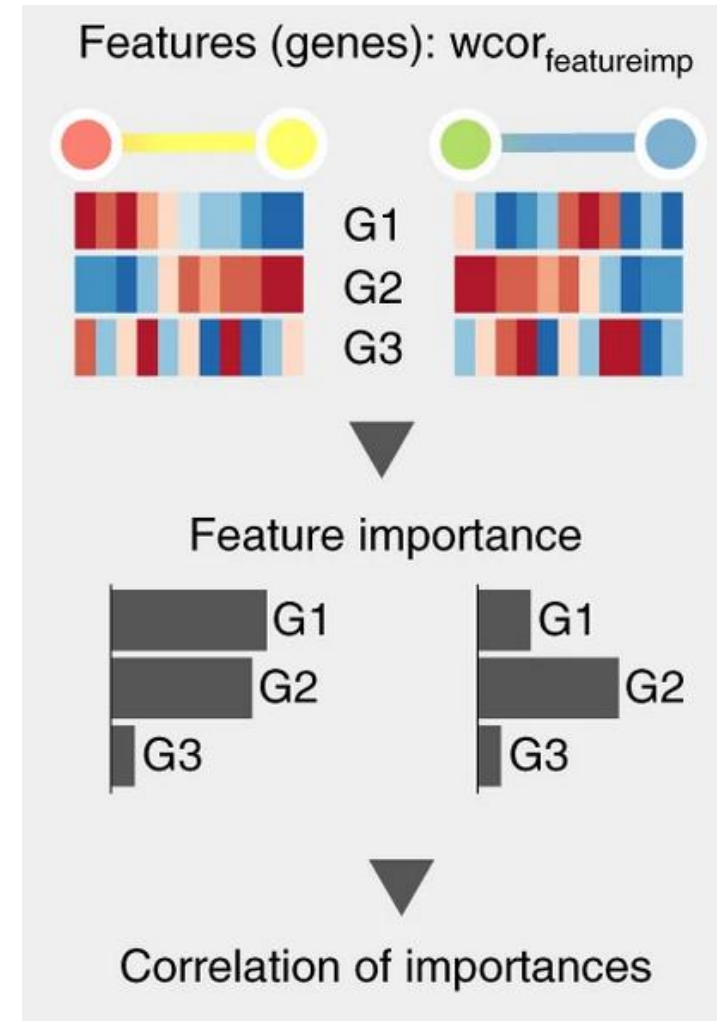
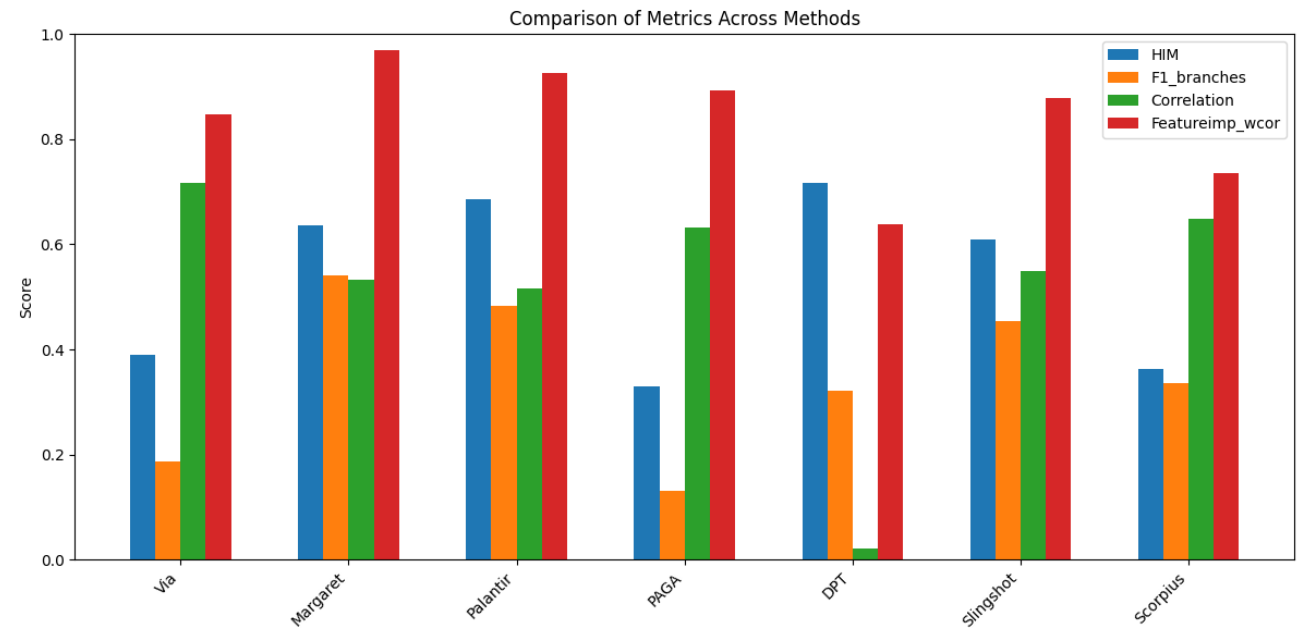


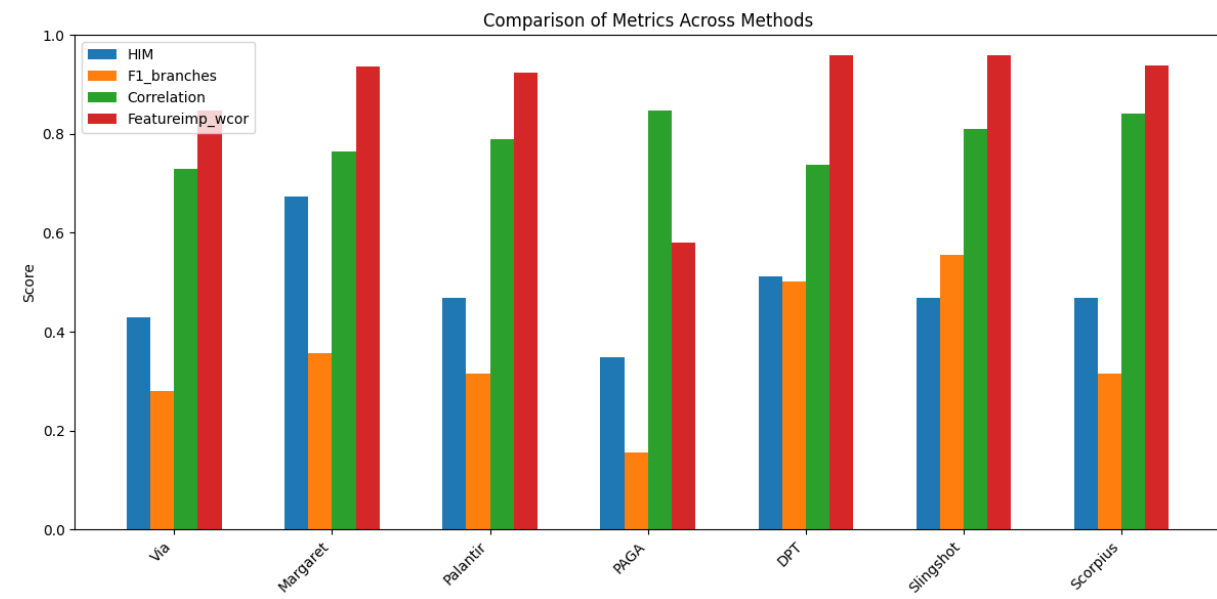
Image source: <https://www.nature.com/articles/s41587-019-0071-9/figures/1>

Results

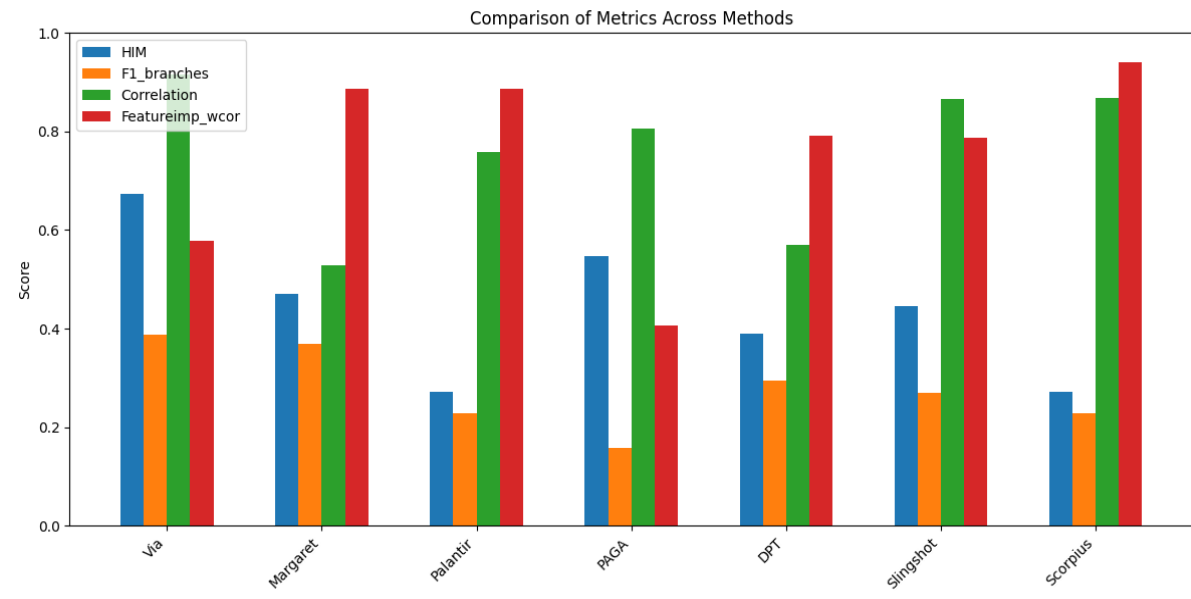
- The ranking of methods varies notably across the four metrics, since each evaluates a different aspect of trajectory inference.
- Some methods do well in topology, others in branch accuracy or gene importance.
- This variation highlights the need for an **overall score** that combines all metrics for balanced evaluation.



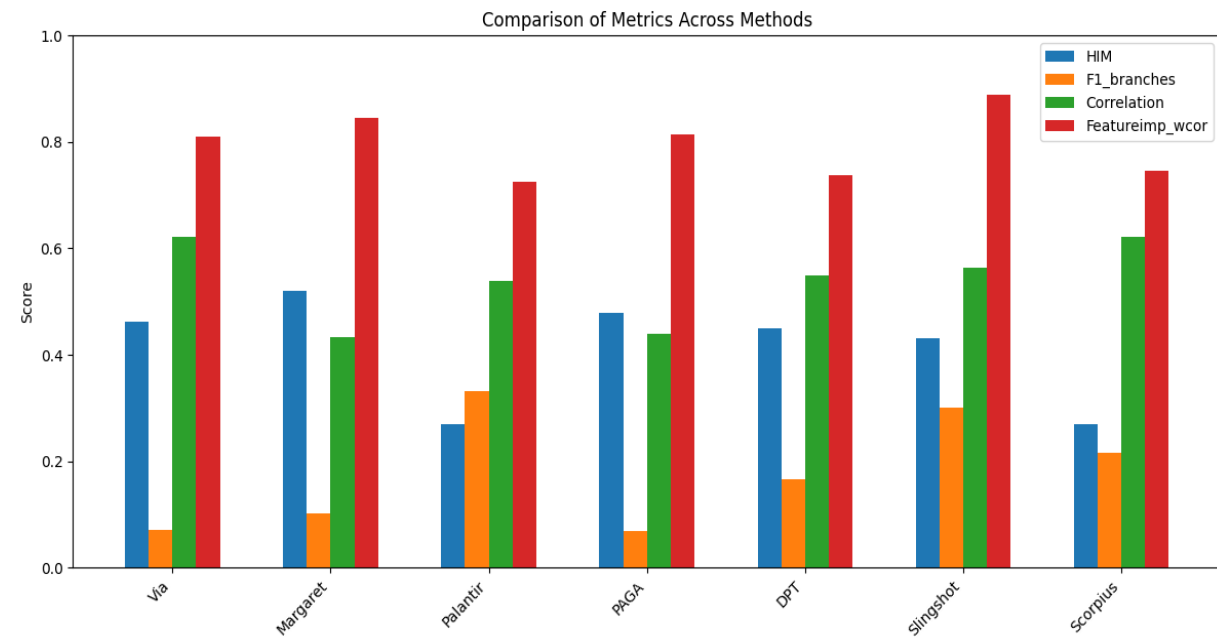
Dataset: Planaria Parenchyma differentiation



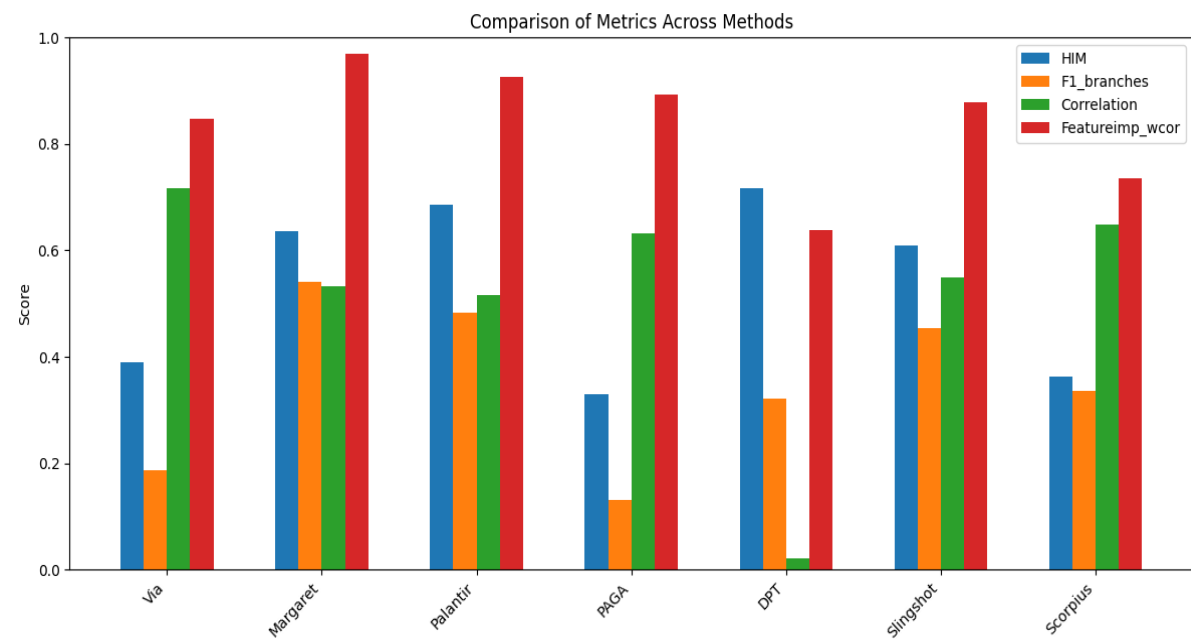
Placenta Trophoblast differentiation



Mouse cell atlas



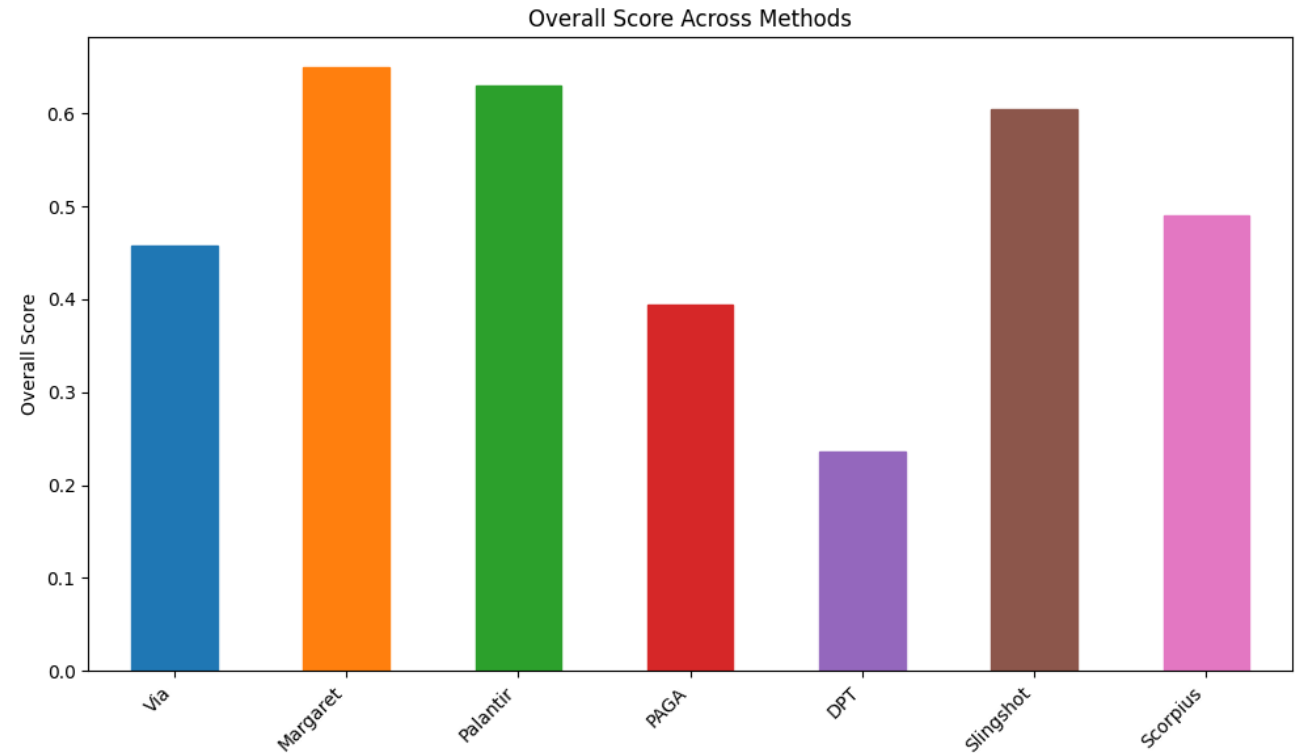
Oligodendrocyte differentiation



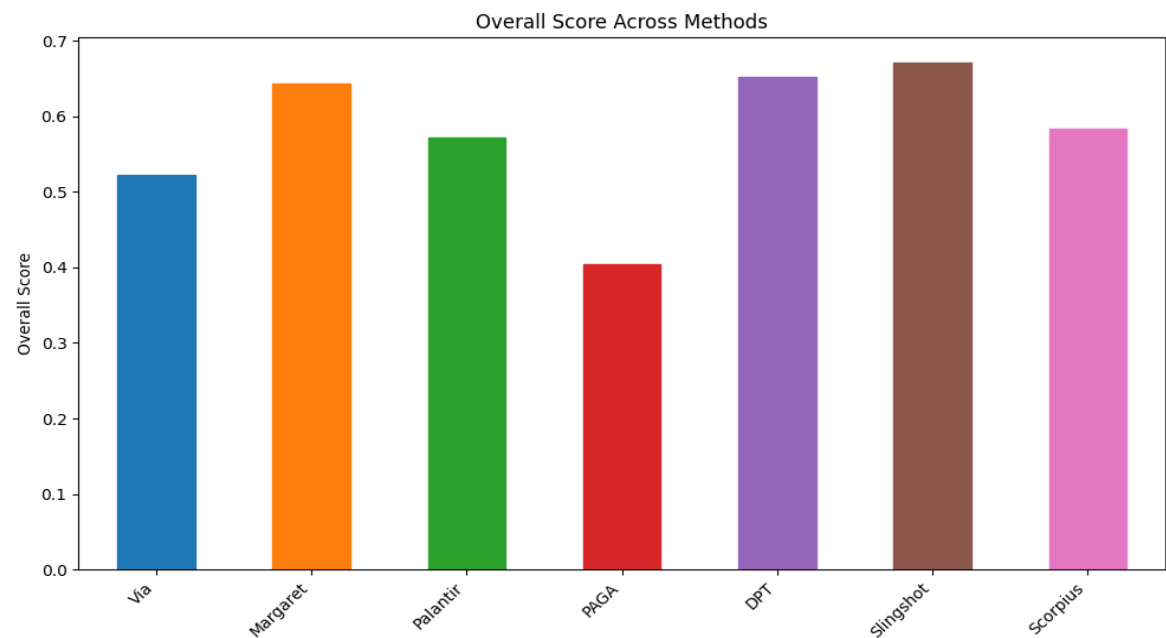
Planaria Parenchyme differentiation

Results

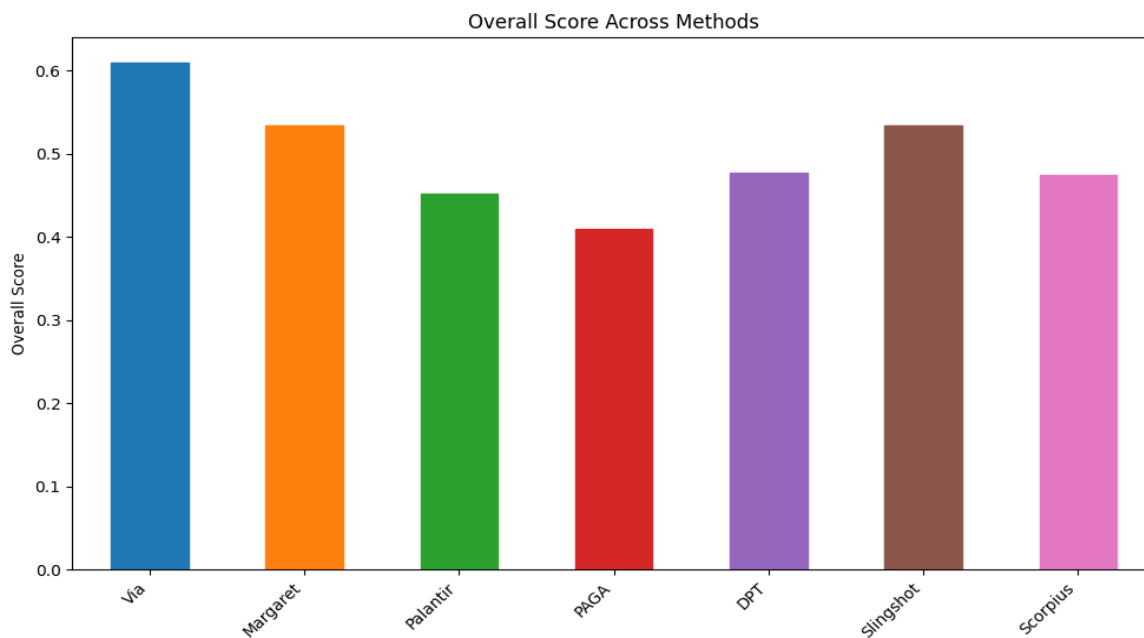
- Overall score is calculated as the **geometric mean** of the metrics HIM, F1_branches, Correlation, FeatureImp_wcor.
- **MARGARET** gives the best overall score followed by Palantir and Slingshot.



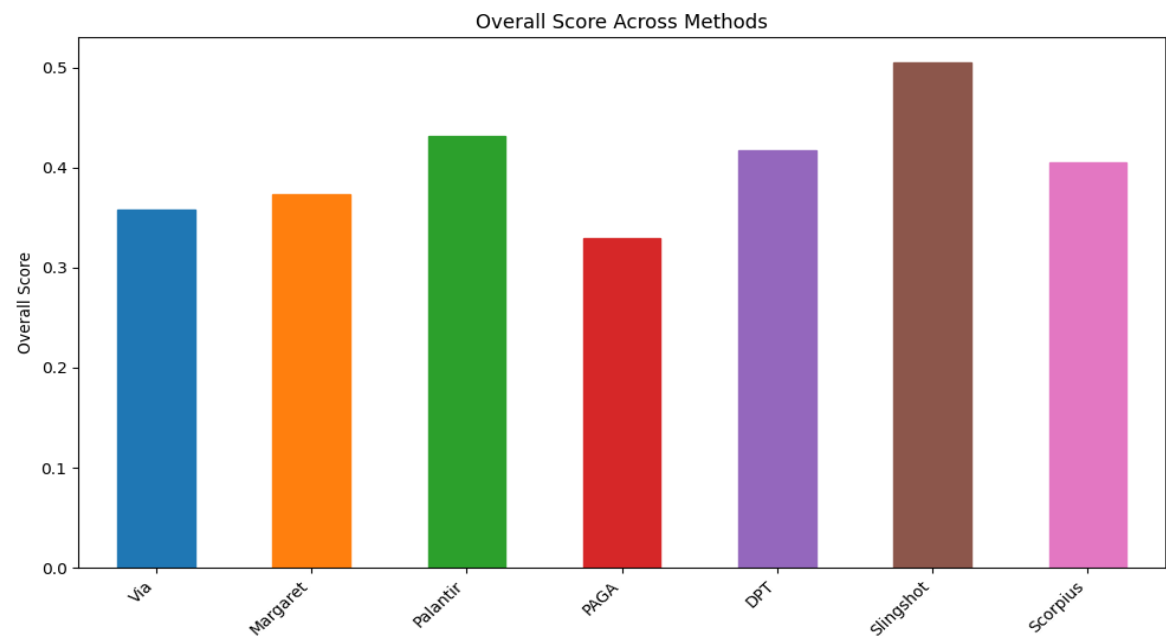
Dataset: Planaria Parenchyma differentiation



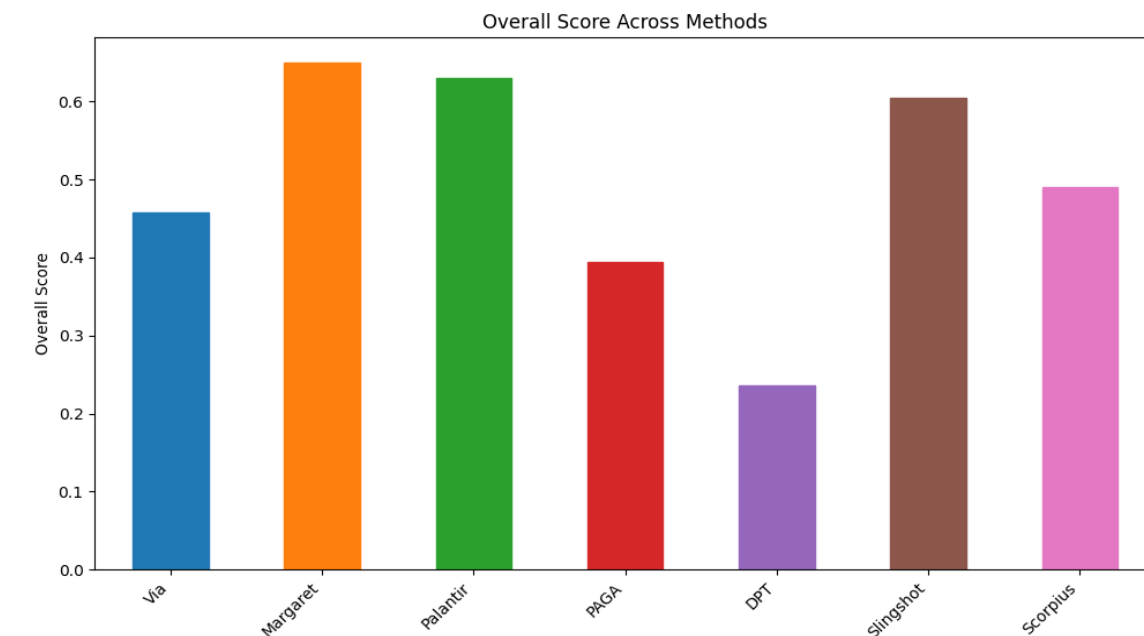
Placenta Trophoblast differentiation



Mouse cell atlas



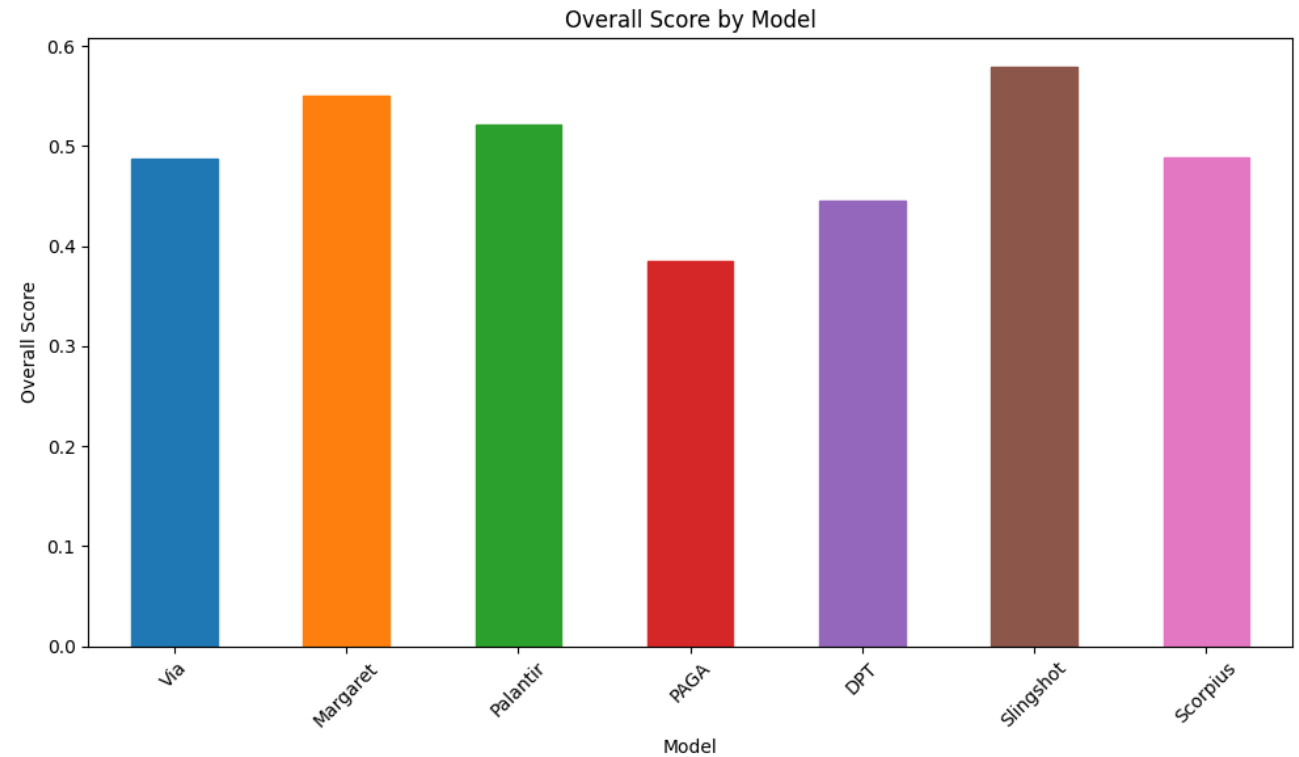
Oligodendrocyte differentiation



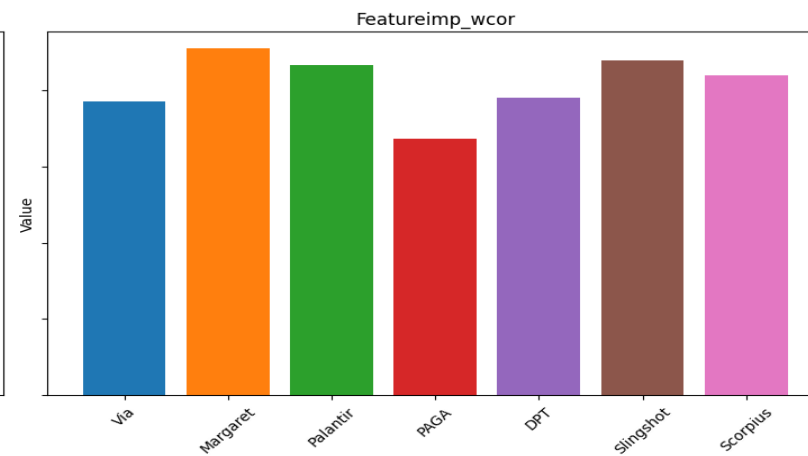
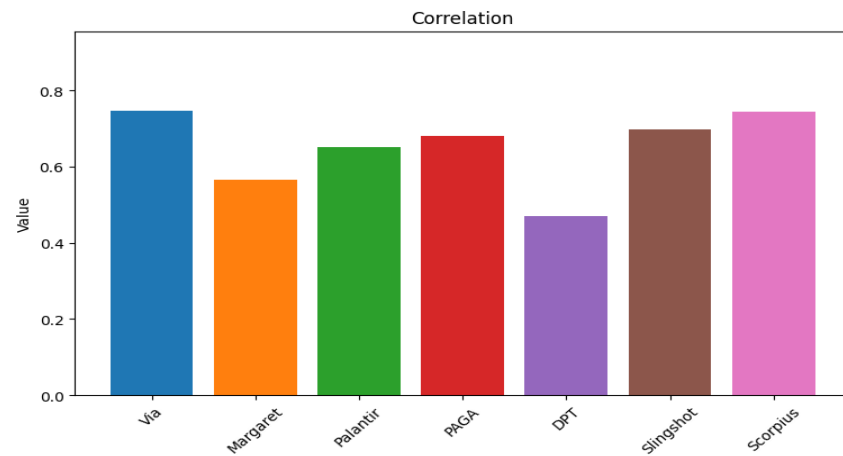
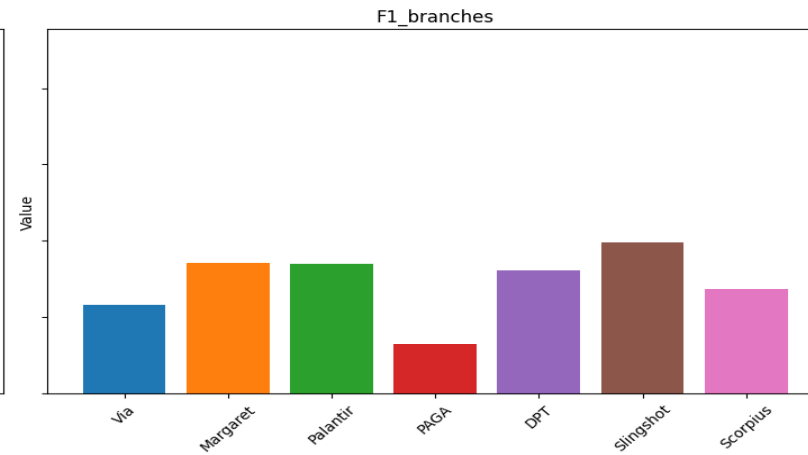
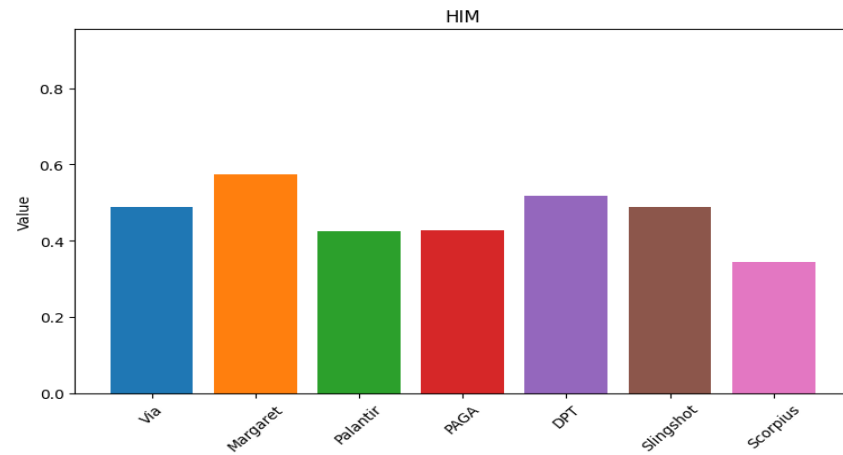
Planaria Parenchyme differentiation

Results

- The net overall scores are calculated as the **arithmetic mean** of the overall scores of the methods across the 4 datasets.
- **Slingshot** gives the best overall score followed by **MARGARET** and **Palantir**.



Results

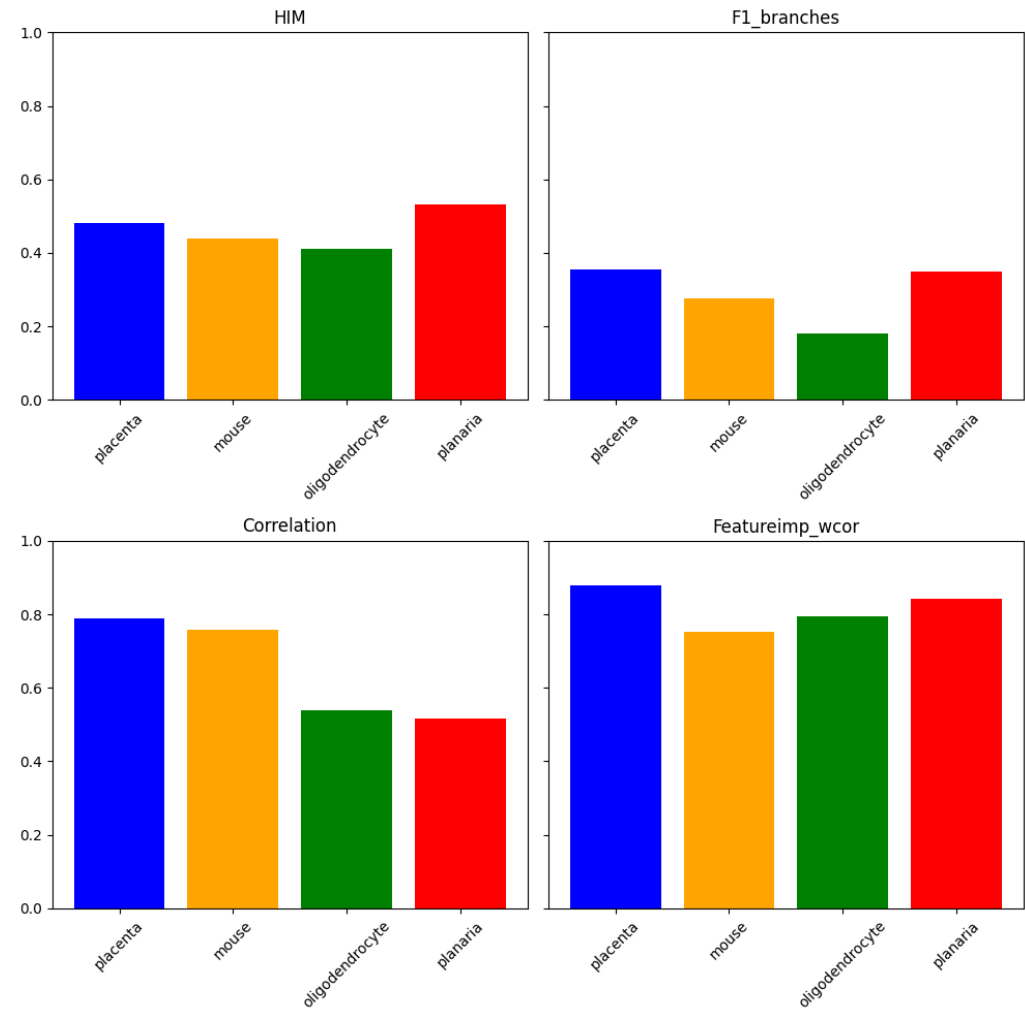


Top Methods in each aspect:

- Topology: **MARGARET**
- Branch assignment: **Slingshot**
- Cell ordering: **VIA**
- Feature importance: **MARGARET**

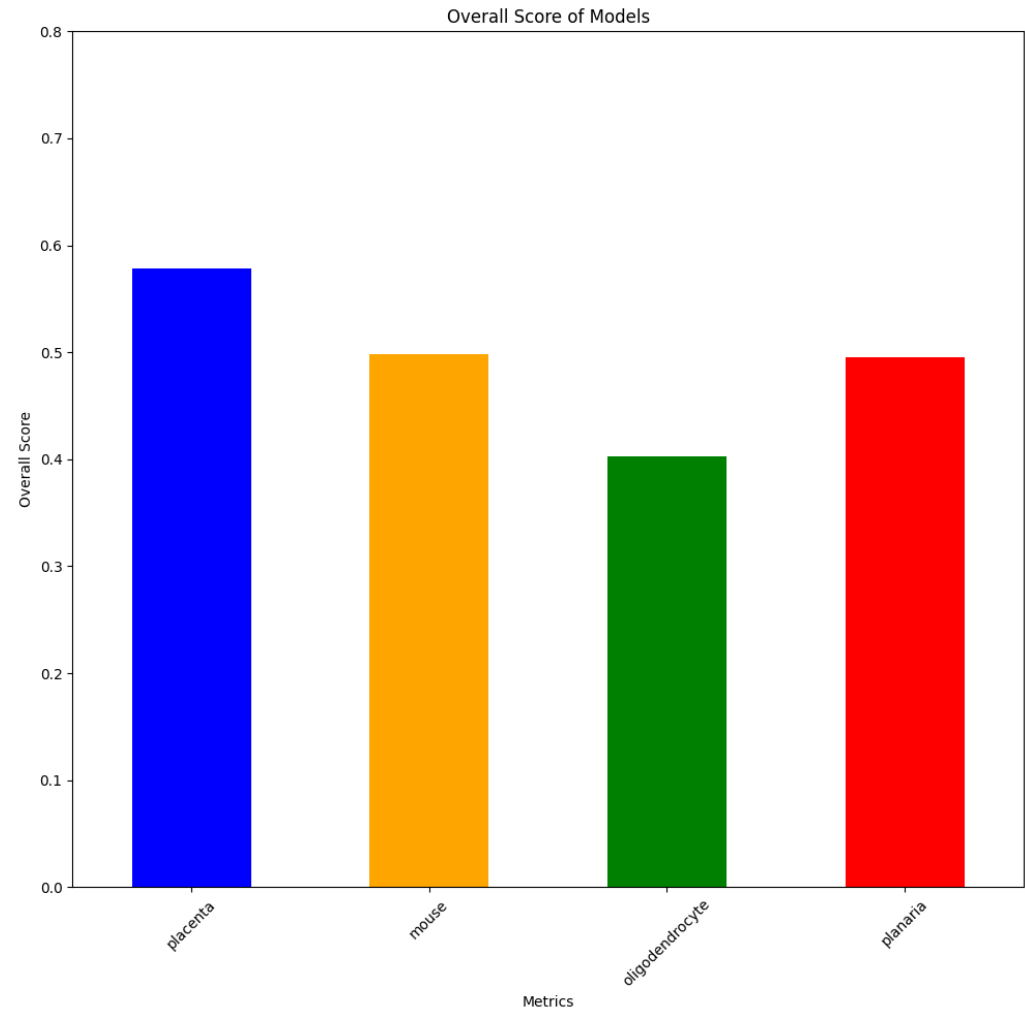
Results

- Each metric for a dataset represents the mean of that metric's values for the methods in the dataset.
- No dataset consistently dominates across all metrics. This indicates that **metrics are not correlated** and capture different aspects of the trajectories
- All datasets show relatively strong scores for featureimp_wcor, suggesting that most methods **preserve gene relevance** well.



Results

- The bars represent the **mean overall score values across all methods** for each dataset.
- For each metric, no single dataset consistently outperforms the others across all metrics.
- This indicates that there is **no systematic bias in the selection of methods** such that they all perform better on one dataset type (e.g., all methods excelling only on linear datasets).





Conclusion

- No method consistently dominates across all datasets and metrics.
- On average across datasets, Slingshot, Margaret, and Palantir perform best.
- The metrics are not strongly correlated and show no systematic bias toward any dataset type.
- Highlights the need to identify which methods suit which dataset types and what factors influence performance
- Future work could be to develop a consensus approach that integrates strengths of multiple methods.

References

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Thank you!