Application of LIGER to integration of seqFISH and scRNA-seq

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Outline

- Overview of LIGER Approach
- Previous Spatial Integration Results
- Comparison of Integration from Different Spatial Transcriptomic Protocols
LIGER Integrates Diverse Single-Cell Datasets

Linked Inference of Genomic Experimental Relationships

- LIGER uses integrative NMF to jointly learns a low-dimensional space
  - Clustering with max factor assignment
  - Quantile normalization
  - Optional Louvain clustering on aligned space
- Allows for the integration of multiple datasets, from
  - Different samples
  - Different species
  - Different modalities

Welch et al., Cell, 2019
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Why Integrate scRNA-seq and Spatial Transcriptomic Data?

<table>
<thead>
<tr>
<th>Spatial Transcriptomics</th>
<th>scRNA-seq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usually few cells</td>
<td>Usually many cells</td>
</tr>
<tr>
<td>Only selected genes</td>
<td>All genes</td>
</tr>
<tr>
<td>In situ measurements</td>
<td>Tissue dissociation required</td>
</tr>
<tr>
<td>Spatial coordinates known</td>
<td>Spatial coordinates lost during dissociation</td>
</tr>
<tr>
<td>No dissociation bias</td>
<td>Dissociation may bias cell type proportions</td>
</tr>
</tbody>
</table>

- Integration with scRNA-seq better resolves cell subtypes
- Spatial transcriptomic data allows imputation of spatial trends and spatial cell type distributions
Previous Work: LIGER Integrates STARmap and scRNA-seq

- Integrated scRNA-seq (71000 cells) and STARmap (2000 cells) from mouse cortex
- Strong alignment between datasets
- Expression of known cell type markers confirmed accurate joint clustering
- Increased resolution for detecting clusters compared to STARmap alone

Welch et al., Cell, 2019

Welch et al. (2019)
Previous Work: LIGER Imputes Spatial Gene Expression

- Averaging of closest scRNA-seq samples imputes spatial distribution of genes not measured in STARmap
- Confirmed accuracy by holding out genes and comparing with Allen Brain Atlas

Welch et al. (2019)
Outline

- Overview of LIGER Approach
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Guiding Questions

1) How do integration results differ by spatial transcriptomic protocol?
2) How does number of shared genes affect results?
Sequential FISH Protocol

- One of the earliest multiplexed FISH approaches
- Sequential rounds of barcoding hybridizations allow measurement of multiple genes using FISH
- Recent versions scale to thousands of genes
**MERFISH Protocol**

- Multiplexed error-robust FISH
- Uses error-correcting binary code to identify each gene
- Recent versions scale to thousands of genes

MERFISH Moffitt et al. (2016)
STARmap Protocol

- Converts tissue into hydrogel
- Rolling circle amplification using SNAIL probes
- Allows measurement of 3D tissue volumes but limited to ~1000 genes

STARmap Wang and Allen et al. (2018)
Comparing Spatial Transcriptomic Protocols

SeqFISH - mouse cortex

STARmap - mouse cortex

MERFISH - mouse hypothalamus
Spatial Transcriptomic + scRNA-seq Integration Strategy

- Completed integrative analysis of given seqFISH data with ViSP subset from Tasic et al. (2018)
- Followed standard LIGER workflow
  - Preprocessing & variable gene selection %>% iNMF %>% quantile norm %>% clustering
  - 14662 scRNA-seq samples with ~43,000 genes and 1597 seqFISH samples with 113 genes
  - 111/113 genes used as features
- Guiding questions
  1) How do integration results differ by spatial transcriptomic protocol?
  2) How does number of genes affect results?
Comparing Integration Results Across Protocols

SeqFISH - mouse cortex  
STARmap - mouse cortex  
MERFISH - mouse hypothalamus
Resolution was improved!
- More, better defined clusters
- Highly aligned integrations allow for imputation of spatial distribution
Suggests that more genes (1020 in STARmap) results in more informative integration
- Most seqFISH cells do not align at all with scRNA data
- Some loss of structure in scRNA data after integration
- ~100 genes is on the lower end, so how many genes are needed?
- Tried repeatedly downsampling STARmap dataset with 1020 genes to determine at what number of genes recorded for the spatial data the integration breaks down
- Depicted here is an analysis with 1020 genes, for which the STARmap and scRNA-seq given clusterings agree, meaning the integration is informative
- Highly aligned after integration
- MERFISH enriched for astrocytes as compared to scRNA-seq
- Found that the increase in quality of spatial sample assignment is approximately linear
- Note that metrics for scRNA-seq samples level out quickly
  - Theorize that at the lowest level, STARmap data obfuscates real patterns in data, whereas with more genes the scRNA-seq is of high enough resolution and quantity to provide structure
- MERFISH data used in analysis came from dataset with 1M samples
- Integration yielded alignment of 0.851 and many distinct clusters
- More informative demonstration of dissociation bias in relative numbers of cells found from each dataset per cluster

Kriebel (2020)
Comparing Integration Results Across Protocols
Acknowledgments

Welch Lab

- Jialin Liu
- April Kriebel

Thank you for your attention!

Kriebel, 2020
References

https://doi.org/10.1016/j.cell.2019.05.006

Data:

