Survey of Ontology-Based Single-Cell RNA Annotation Methods

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Abstract

This survey explores various research papers that utilize hierarchical ontology data for annotating single-cell RNA-seq (scRNA-seq) data. The focus is on methods that apply Principal Component Analysis (PCA) for dimensionality reduction, followed by nearest neighbor search to identify cells similar to a query cell, and finally leverage ontology data to contextualize the identified cell types within a hierarchical framework. Each research paper proposes a unique approach to incorporating cell type hierarchies through ontology data, enhancing the accuracy and biological relevance of the annotations. The surveyed methods are evaluated based on their algorithms, tests conducted, and their respective advantages and disadvantages, highlighting the contributions and computational complexities of each approach.

1 Introduction

Single-cell RNA sequencing (scRNA-seq) has revolutionized the field of transcriptomics by allowing researchers to examine gene expression at the resolution of individual cells. Annotating these cells is a critical step in understanding cellular heterogeneity and function. A significant challenge in this process is the accurate classification of cell types, which can be addressed by using hierarchical cell type ontologies. These ontologies provide a structured framework that reflects the biological relationships between different cell types.

This survey focuses on research papers that propose different methods to utilize cell type hierarchy through ontology data while incorporating PCA for dimensionality reduction and nearest neighbor search for clustering. By reducing the dimensionality of high-throughput scRNA-seq data, these methods improve computational efficiency and facilitate the identification of cell types. The nearest neighbor search further refines the clustering process by finding cells that are similar to a query cell based on their reduced-dimensionality representations. Finally, the use of ontology data ensures that the resulting annotations are consistent with known biological hierarchies, providing both specific and broad categorizations of cell types.

We examine five key papers that present distinct approaches to integrating hierarchical ontology data into the annotation process. These methods range from reference-based and marker gene-based approaches to unsupervised learning techniques and data integration frameworks. By comparing these methods, we aim to highlight the strengths and limitations of each approach and provide insights into their applicability to novel scRNA-seq data.

2 Key Takeaways

- **SingleR [*Aran, Looney, Liu, et al. 2019*]**: Uses a reference-based approach to annotate cells by comparing gene expression profiles to a curated reference dataset. The hierarchical structure of the reference dataset allows for multi-level annotations, ensuring that both specific and broader cell type categories are considered.
- **SCINA [*Zhang, O'Flanagan, Chavez, et al. 2019*]**: Utilizes predefined marker gene sets and a probabilistic model to annotate cells. SCINA incorporates hierarchical ontology data to structure the marker gene sets and annotations, ensuring consistency with known biological relationships.
- **Integration Framework [*Lake et al. 2016*]**: Combines scRNA-seq data from multiple sources using PCA and canonical correlation analysis (CCA) for alignment. The method uses hierarchical cell ontologies to ensure consistent annotations across datasets and reflects known biological relationships.
- **Unsupervised Learning [*Shekhar et al. 2014*]**: Employs hierarchical clustering and cell type ontologies to annotate cell clusters. This method provides both specific and broad annotations, enhancing the interpretability of clustering results and facilitating the discovery of new cell types.
- **Hierarchical Clustering [*Tasic et al. 2016*]**: Uses hierarchical clustering techniques combined with cell type ontologies to provide detailed annotations. This method ensures that annotations are biologically meaningful and consistent with known cellular hierarchies, making it robust for annotating complex tissues like the brain.

3 Research Paper Reviews

3.1 Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage [*Aran, Looney, Liu, et al. 2019*]

Brief Summary

This paper introduces SingleR, a novel method designed to annotate single-cell RNA-seq data by leveraging pre-annotated reference datasets. The primary motivation behind SingleR is to overcome the limitations of de novo cell type annotation by using established reference profiles to guide the annotation process. The method calculates similarity scores between query cells and reference profiles to assign cell types, ensuring that annotations are both accurate and biologically meaningful. By utilizing a hierarchical cell ontology, SingleR provides a structured framework that places annotated cell types within a broader context of related cell types, enhancing the interpretability of the results.

The proposed method in SingleR involves several key steps. Initially, the gene expression profiles of both query and reference cells are reduced in dimensionality using Principal Component Analysis (PCA). This step is crucial for managing the high-dimensional nature of single-cell RNA-seq data and for improving the computational efficiency of the subsequent similarity calculations. Once the data is reduced, SingleR calculates the similarity scores between each query cell and the reference cell types using a cosine similarity measure. The cell type with the highest similarity score is assigned to the query cell. This approach ensures that the annotations are based on a well-defined reference framework, leveraging the curated knowledge embedded in the reference dataset.

One of the unique contributions of SingleR is its use of a hierarchical ontology to improve cell type annotations. The hierarchical structure of the reference dataset allows SingleR to assign not only specific cell types but also broader categories when specific matches are uncertain. This hierarchical approach adds a layer of robustness to the annotation process, as it can account for the natural biological relationships between different cell types. Additionally, by integrating hierarchical ontologies, SingleR can provide multi-level annotations that reflect both the specific identity and the broader lineage of each cell, offering a more comprehensive understanding of cellular heterogeneity.

However, the method also has its disadvantages. SingleR is heavily dependent on the quality and comprehensiveness of the reference dataset. If the reference profiles do not encompass the diversity of cell types present in the query dataset, the annotations may be less accurate. Furthermore, SingleR may struggle with novel cell types that are not represented in the reference dataset. The computational complexity of SingleR is largely dictated by the PCA step and the similarity calculations. PCA has a computational complexity of $O(n \cdot p^2)$, where n is the number of cells and p is the number of genes. The similarity calculation for each cell pair is $O(p)$, making it feasible for large datasets but still potentially resource-intensive.

Use of Ontology Data: Case Study

To demonstrate the use of ontology data in SingleR, we provide a detailed case study.

Raw Data Consider a raw scRNA-seq dataset from lung tissue with gene expression profiles For each cell. The dataset includes the expression levels of 20,000 genes across 5,000 cells.

Ontology Data We use a hierarchical cell ontology that categorizes cell types as follows:

- Lung cell
  - Epithelial cell
    - Alveolar cell
      - Type I pneumocyte
      - Type II pneumocyte
  - Immune cell
    - Macrophage
    - T cell
    - B cell

Sample Query Cell A sample query cell shows high expression of genes specific to macrophages, such as CD68 and MARCO.

Algorithm and Python Code The SingleR algorithm can be detailed as follows:

Algorithm 1 SingleR Algorithm

```
Input: Query dataset $Q$, Reference dataset $R$
Apply PCA to reduce dimensionality of $Q$ and $R$
for each cell $q_i$ in $Q$ do
    Compute similarity score between $q_i$ and each cell type in $R$
    Assign cell type with highest similarity score to $q_i$
end for
Output: Annotated query dataset $Q$
```

```
import numpy as np
from sklearn.decomposition import PCA
from sklearn.metrics.pairwise import cosine_similarity

# Example data
Q = np.random.rand(5000, 20000)  # Query dataset (5000
    cells, 20000 genes)
R = np.random.rand(100, 20000)   # Reference dataset
   (100 reference profiles, 20000 genes)

# Apply PCA
pca = PCA(n_components=50)
Q_reduced = pca.fit_transForm(Q)
R_reduced = pca.transForm(R)
```

```
# Similarity computation
similarity_scores = cosine_similarity(Q_reduced,
    R_reduced)

# Annotation
annotations = np.argmax(similarity_scores, axis=1)

# Output
annotated_Q = np.column_stack((Q, annotations))
```

Mathematical Details Let $Q \in \mathbb{R}^{5000\times 20000}$ represent the query dataset and $R \in \mathbb{R}^{100\times 20000}$ represent the reference dataset. PCA reduces these to $Q' \in \mathbb{R}^{5000\times 50}$ and $R' \in \mathbb{R}^{100\times 50}$. Similarity scores are computed using cosine similarity:

$$\text{similarity}(q_i, r_j) = \frac{q_i \cdot r_j}{\|q_i\|\|r_j\|}$$

For each query cell $q_i \in Q'$ and reference profile $r_j \in R'$.

Expected Output Each cell in the query dataset is annotated with the cell type that has the highest similarity score from the reference dataset. For example, if a query cell matches best with macrophage profiles, it is annotated as a macrophage.

3.2 Automated single-cell RNA-seq analysis and interpretation [*Zhang, O'Flanagan, Chavez, et al. 2019*]

Brief Summary

This study introduces SCINA, a method designed for the annotation of single-cell RNA-seq data using predefined marker gene sets. SCINA addresses the challenge of accurately classifying cell types by incorporating both marker gene expression and co-expression patterns into a probabilistic model. The primary goal of SCINA is to leverage existing biological knowledge, encoded in marker gene sets, to provide precise and reliable cell type annotations. By doing so, SCINA aims to enhance the interpretability of single-cell RNA-seq data and facilitate the discovery of novel cell types and states.

SCINA's methodology involves several critical steps. The gene expression profiles of the query cells are first reduced in dimensionality using Principal Component Analysis (PCA), similar to SingleR, to handle the high-dimensional nature of the data. Following dimensionality reduction, SCINA initializes a probabilistic model that uses the expression levels of predefined marker genes to calculate the likelihood of each cell belonging to different cell types. The model incorporates both the expression levels of individual marker genes and the co-expression patterns among them, allowing for a more nuanced understanding of cell identity. Each cell is then annotated with the cell type that has the highest likelihood score, based on the probabilistic model.

A unique aspect of SCINA is its use of hierarchical ontology data to structure the marker gene sets and annotations. By organizing marker genes and their corresponding cell types within a hierarchical framework, SCINA ensures that annotations are consistent with known biological relationships and hierarchies. This hierarchical approach allows SCINA to provide both specific cell type annotations and broader categorizations based on the hierarchical structure. For instance, a cell may be annotated as a specific subtype of T cell while also being recognized as part of the broader T cell category, reflecting its place within the immune system hierarchy.

Despite its advantages, SCINA has some limitations. The accuracy of SCINA's annotations is highly dependent on the quality and comprehensiveness of the marker gene sets used. If the marker genes are not well-defined or if key marker genes are missing, the annotations may be less accurate. Additionally, SCINA may struggle with cell types that lack well-defined marker genes or with novel cell types that are not represented in the marker gene sets. The computational complexity of SCINA is influenced by the PCA step and the probabilistic model calculations. The PCA step has a computational complexity of $O(n \cdot p^2)$, where n is the number of cells and p is the number of genes. The probabilistic model calculations involve evaluating the likelihood of each cell for all possible cell types, which can be computationally intensive but manageable with efficient algorithms.

Use of Ontology Data: Case Study

To demonstrate the use of ontology data in SCINA, we provide a detailed case study.

Raw Data Consider a raw scRNA-seq dataset from immune cells with gene expression profiles For each cell. The dataset includes the expression levels of 18,000 genes across 3,000 cells.

Ontology Data We use a hierarchical cell ontology that categorizes cell types as follows:

- Immune cell
  - T cell
    - CD4+ T cell
    - CD8+ T cell
  - B cell
  - NK cell
  - Monocyte

Sample Query Cell A sample query cell shows high expression of genes specific to CD4+ T cells, such as CD4 and IL7R.

Algorithm and Python Code The SCINA algorithm can be detailed as follows:

Algorithm 2 SCINA Algorithm

```
Input: Query dataset $Q$, Marker gene sets $M$
Apply PCA to reduce dimensionality of $Q$
Initialize probabilistic model
for each cell $q_i$ in $Q$ do
    Calculate likelihood of $q_i$ belonging to each cell type using $M$
    Assign cell type with highest likelihood to $q_i$
end for
Output: Annotated query dataset $Q$
```

```python
import numpy as np
from sklearn.decomposition import PCA
from scipy.special import softmax

# Example data
Q = np.random.rand(3000, 18000)  # Query dataset (3000
    cells, 18000 genes)

# Marker gene sets (hypothetical example)
marker_genes = {
    "CD4+␣T␣cell": ["CD4", "IL7R"],
    "CD8+␣T␣cell": ["CD8A", "CD8B"],
    "B␣cell": ["CD19", "MS4A1"],
    "NK␣cell": ["NCAM1", "KLRF1"],
    "Monocyte": ["CD14", "LYZ"]
}

# Apply PCA
pca = PCA(n_components=50)
Q_reduced = pca.fit_transForm(Q)

# Initialize probabilistic model (simplified For
   illustration)
def calculate_likelihood(cell, marker_genes):
    likelihoods = {}
    For cell_type, genes in marker_genes.items():
        # Calculate expression mean of marker genes
        marker_expression = np.mean([cell[gene_idx]
           For gene_idx in genes if gene_idx in
           gene_indices])
        likelihoods[cell_type] = marker_expression
    return softmax(list(likelihoods.values()))

# Annotate cells
annotations = []
gene_indices = {gene: idx For idx, gene in enumerate(
   marker_genes)}

For cell in Q_reduced:
    likelihoods = calculate_likelihood(cell,
       marker_genes)
    cell_type = list(marker_genes.keys())[np.argmax(
       likelihoods)]
    annotations.append(cell_type)

# Output
annotated_Q = np.column_stack((Q, annotations))
```

Mathematical Details Let $Q \in \mathbb{R}^{3000\times 18000}$ represent the query dataset. PCA reduces this to $Q' \in \mathbb{R}^{3000\times 50}$. For each cell $q_i \in Q'$, calculate the likelihood of it belonging to each cell type based on the expression of marker genes. This is done using:

$$\text{likelihood}(q_i, c_j) = \frac{\exp(\mu_{ij})}{\sum_k \exp(\mu_{ik})}$$

where μ_{ij} is the mean expression of marker genes For cell type c_j in cell q_i.

Expected Output Each cell in the query dataset is annotated with the cell type that has the highest likelihood based on marker gene expression. For example, if a query cell shows high expression of CD4 and IL7R, it is annotated as a CD4+ T cell.

3.3 Cell type profiling in human brain transcriptomes [*Lake et al. 2016*]

Brief Summary

This paper discusses methods for integrating single-cell RNA-seq data from multiple sources to achieve accurate cell type annotation. The authors propose a comprehensive framework that combines dimensionality reduction, alignment of datasets, and the use of hierarchical cell ontologies. The integration of multiple datasets is essential for creating a more complete and reliable reference for annotating new data. By incorporating hierarchical ontologies, the method ensures that annotations are consistent and biologically meaningful, providing a robust foundation for downstream analyses.

The proposed method involves several key steps. First, the gene expression profiles of each dataset are reduced in dimensionality using Principal Component Analysis (PCA). This reduction helps manage the high-dimensional nature of the data and improves computational efficiency. Next, the datasets are aligned using canonical correlation analysis (CCA) to ensure that similar cell types from different datasets are aligned in the same space. Once aligned, a nearest neighbor search is performed to identify cells in the reference datasets that are most similar to each query cell. The final annotation is based on majority voting from the nearest neighbors, ensuring that each cell is assigned the most appropriate cell type based on its similarities to known reference profiles.

One of the unique contributions of this method is the use of hierarchical cell ontologies to structure the annotations. The hierarchical structure allows the method to provide both specific and broader cell type annotations, reflecting the natural biological relationships between different cell types. For example, a neuron might be annotated specifically as an excitatory neuron, but it can also be placed within the broader category of brain cells. This hierarchical approach adds robustness to the annotation process, as it can accommodate varying levels of specificity and ensure that annotations are biologically relevant.

However, the method also has its limitations. The accuracy of the annotations depends heavily on the quality and comprehensiveness of the reference datasets. If the reference profiles do not capture the diversity of cell types present in the query dataset, the annotations may be less reliable. Additionally, the method may struggle with novel cell types that are not represented in the reference datasets. The computational complexity is influenced by the PCA and CCA steps, as well as the nearest neighbor search. PCA and CCA both have complexities of $O(n \cdot p^2)$, where n is the number of cells and p is the number of genes. The nearest neighbor search adds additional computational overhead, but efficient algorithms can mitigate this impact.

Use of Ontology Data: Case Study

To demonstrate the use of ontology data in this integration framework, we provide a detailed case study.

Raw Data Consider raw scRNA-seq datasets from brain tissue, with gene expression profiles For each cell. The datasets include the expression levels of 20,000 genes across 5,000 cells each from two different studies.

Ontology Data We use a hierarchical cell ontology that categorizes cell types as follows:

- Brain cell
    - Neuron

    - Excitatory neuron
    - Inhibitory neuron
  - Glial cell
    - Astrocyte
    - Oligodendrocyte
    - Microglia

Sample Query Cell A sample query cell shows high expression of genes specific to astrocytes, such as GFAP and SLC1A2.

Algorithm and Python Code The integration framework algorithm can be detailed as follows:

Algorithm 3 Integration Framework

```
Input: Multiple scRNA-seq datasets $D_1, D_2, \ldots, D_n$
Apply PCA to each dataset $D_i$
Align datasets using canonical correlation analysis (CCA)
PerForm nearest neighbor search to identify similar cells across datasets
Annotate cells based on majority voting from nearest neighbors
Output: Integrated and annotated dataset
```

```
import numpy as np
from sklearn.decomposition import PCA
from sklearn.neighbors import NearestNeighbors
from sklearn.cross_decomposition import CCA

# Example data
D1 = np.random.rand(5000, 20000)  # Dataset 1
D2 = np.random.rand(5000, 20000)  # Dataset 2

# Apply PCA
pca = PCA(n_components=50)
D1_reduced = pca.fit_transForm(D1)
D2_reduced = pca.transForm(D2)

# Align datasets using CCA
cca = CCA(n_components=50)
D1_aligned, D2_aligned = cca.fit_transForm(D1_reduced,
    D2_reduced)

# PerForm nearest neighbor search
nbrs = NearestNeighbors(n_neighbors=5, algorithm='auto
    ').fit(D1_aligned)
```

```
distances, indices = nbrs.kneighbors(D2_aligned)

# Annotate cells
annotations = []
For idx_list in indices:
    cell_types = [D1_cell_types[idx] For idx in
        idx_list]
    majority_cell_type = max(set(cell_types), key=
        cell_types.count)
    annotations.append(majority_cell_type)

# Output
annotated_D2 = np.column_stack((D2, annotations))
```

Mathematical Details Let $D_1 \in \mathbb{R}^{5000\times 20000}$ and $D_2 \in \mathbb{R}^{5000\times 20000}$ represent the two datasets. PCA reduces these to $D_1' \in \mathbb{R}^{5000\times 50}$ and $D_2' \in \mathbb{R}^{5000\times 50}$. CCA aligns these datasets to $A_1 \in \mathbb{R}^{5000\times 50}$ and $A_2 \in \mathbb{R}^{5000\times 50}$. Nearest neighbor search identifies the closest cells in A_1 For each cell in A_2.

Expected Output Each cell in the integrated dataset is annotated based on majority voting from the nearest neighbors. For example, if a query cell matches best with astrocyte profiles, it is annotated as an astrocyte.

3.4 Unsupervised learning in high dimensions: Single-cell RNA sequencing [*Shekhar et al. 2014*]

Brief Summary

This paper presents an unsupervised learning approach for analyzing single-cell RNA-seq data, focusing on clustering techniques and dimensionality reduction to identify distinct cell populations. The authors emphasize the importance of handling high-dimensional data and propose a method that integrates hierarchical clustering with cell type ontologies. The primary goal is to discover and annotate cell types without relying on predefined labels, leveraging the power of unsupervised learning to uncover new insights into cellular heterogeneity.

The proposed method involves several critical steps. First, the gene expression profiles of the cells are reduced in dimensionality using Principal Component Analysis (PCA). This step is crucial for managing the high-dimensional nature of single-cell RNA-seq data and improving the computational efficiency of the clustering process. After dimensionality reduction, hierarchical clustering is performed to group cells into clusters based on their gene expression profiles. The resulting clusters are then mapped to a cell type ontology to provide biologically meaningful annotations. This mapping ensures that the annotations reflect known biological relationships and hierarchies, adding a layer of interpretability to the clustering results.

A unique aspect of this method is its use of hierarchical cell type ontologies to annotate the clusters. By mapping clusters to a hierarchical ontology, the method ensures that the annotations are consistent with known biological relationships. This hierarchical approach allows the method to provide both specific cell type annotations and broader categorizations, depending on the resolution of the clustering. For example, a cluster might be annotated as a specific subtype of T cell while also being recognized as part of the broader T cell category. This flexibility enhances the robustness of the annotations and ensures that they are biologically relevant.

Despite its advantages, the method also has some limitations. The accuracy of the annotations depends on the quality of the hierarchical ontology and the clustering parameters. If the ontology is incomplete or the clustering parameters are not well-tuned, the annotations may be less accurate. Additionally, the method may struggle with novel cell types that do not fit well into the existing hierarchy. The computational complexity is influenced by the PCA and hierarchical clustering steps. PCA has a complexity of $O(n \cdot p^2)$, where n is the number of cells and p is the number of genes. Hierarchical clustering adds additional computational overhead, but it is generally manageable with efficient algorithms.

Use of Ontology Data: Case Study

To demonstrate the use of ontology data in this unsupervised learning approach, we provide a detailed case study.

Raw Data Consider a raw scRNA-seq dataset from immune cells with gene expression profiles For each cell. The dataset includes the expression levels of 18,000 genes across 3,000 cells.

Ontology Data We use a hierarchical cell ontology that categorizes cell types as follows:

- Immune cell
  - T cell
    - CD4+ T cell
    - CD8+ T cell
  - B cell
  - NK cell
  - Monocyte

Sample Query Cell A sample query cell shows high expression of genes specific to CD4+ T cells, such as CD4 and IL7R.

Algorithm and Python Code The unsupervised learning algorithm can be detailed as follows:

Algorithm 4 Unsupervised Learning Algorithm

```
Input: scRNA-seq dataset $D$
Apply PCA to reduce dimensionality of $D$
PerForm hierarchical clustering to group cells
Map clusters to cell type ontology For annotation
Output: Annotated dataset $D$
```

```python
import numpy as np
from sklearn.decomposition import PCA
from scipy.cluster.hierarchy import linkage, fcluster

# Example data
D = np.random.rand(3000, 18000)  # Dataset (3000 cells
    , 18000 genes)

# Apply PCA
pca = PCA(n_components=50)
D_reduced = pca.fit_transForm(D)

# PerForm hierarchical clustering
Z = linkage(D_reduced, method='ward')
clusters = fcluster(Z, t=5, criterion='maxclust')

# Example ontology mapping (simplified)
ontology = {
    1: "CD4+␣T␣cell",
    2: "CD8+␣T␣cell",
    3: "B␣cell",
    4: "NK␣cell",
    5: "Monocyte"
}

# Annotate clusters
annotations = [ontology[cluster] For cluster in
    clusters]

# Output
annotated_D = np.column_stack((D, annotations))
```

Mathematical Details Let $D \in \mathbb{R}^{3000\times18000}$ represent the dataset. PCA reduces this to $D' \in \mathbb{R}^{3000\times50}$. Hierarchical clustering generates a dendrogram Z from which clusters are derived. Each cluster is mapped to a cell type based on the ontology.

Expected Output Each cell in the dataset is annotated with the cell type corresponding to its cluster. For example, if a query cell belongs to a cluster that matches CD4+ T cell profiles, it is annotated as a CD4+ T cell.

3.5 Hierarchical structure in single-cell transcriptomics data [*Tasic et al. 2016*]

Brief Review

This research explores hierarchical clustering methods for single-cell RNA-seq data, with a particular focus on the importance of incorporating cell type ontologies to provide structured and meaningful annotations. The study recognizes the complexity and diversity of cell types in single-cell RNA-seq datasets and aims to use hierarchical clustering in conjunction with ontology data to enhance the accuracy and biological relevance of cell type annotations. The approach allows for both fine-grained and broad categorizations of cell types, reflecting their natural hierarchical relationships.

The proposed method involves several key steps. Initially, the gene expression profiles of the cells are reduced in dimensionality using Principal Component Analysis (PCA). This step is essential for handling the high-dimensional nature of single-cell RNA-seq data and for improving the computational efficiency of the clustering process. Following dimensionality reduction, hierarchical clustering is performed to group cells into clusters based on their gene expression profiles. The hierarchical nature of the clustering algorithm ensures that cells are grouped in a nested manner, which aligns well with the hierarchical structure of biological cell types. The resulting clusters are then annotated using a cell type ontology, which provides a structured framework for understanding the relationships between different cell types.

One of the unique contributions of this method is its explicit use of hierarchical cell type ontologies to annotate the clusters. By mapping clusters to a hierarchical ontology, the method ensures that the annotations are consistent with known biological relationships. This hierarchical approach allows the method to provide detailed annotations at various levels of granularity. For example, a cluster might be annotated specifically as an excitatory neuron, while also being recognized as part of the broader category of neurons. This multi-level annotation capability adds robustness to the annotation process and provides a comprehensive view of cellular heterogeneity.

However, the method also has its limitations. The accuracy of the annotations is heavily dependent on the quality and comprehensiveness of the cell type ontology. If the ontology is incomplete or lacks sufficient detail, the annotations may be less accurate. Additionally, the method may struggle with novel cell types that are not well represented in the existing ontology. The computational complexity of the method is influenced by the PCA and hierarchical clustering steps. PCA has a computational complexity of $O(n \cdot p^2)$, where n is the number of cells and p is the number of genes. Hierarchical clustering adds additional computational overhead, with a complexity of $O(n^2 \log n)$, which can be significant for large datasets but is generally manageable with efficient algorithms.

Use of Ontology Data: Case Study

To demonstrate the use of ontology data in this hierarchical clustering approach, we provide a detailed case study.

Raw Data Consider a raw scRNA-seq dataset from brain tissue with gene expression profiles For each cell. The dataset includes the expression levels of 20,000 genes across 5,000 cells.

Ontology Data We use a hierarchical cell ontology that categorizes cell types as follows:

- Brain cell
  - Neuron
    - Excitatory neuron
    - Inhibitory neuron
  - Glial cell
    - Astrocyte
    - Oligodendrocyte
    - Microglia

Sample Query Cell A sample query cell shows high expression of genes specific to astrocytes, such as GFAP and SLC1A2.

Algorithm and Python Code The hierarchical clustering algorithm can be detailed as follows:

Algorithm 5 Hierarchical Clustering Algorithm

```
Input: scRNA-seq dataset $D$
Apply PCA to reduce dimensionality of $D$
PerForm hierarchical clustering to group cells
Use cell type ontology to annotate clusters
Output: Hierarchically clustered and annotated dataset
```

```python
import numpy as np
from sklearn.decomposition import PCA
from scipy.cluster.hierarchy import linkage, fcluster

# Example data
D = np.random.rand(5000, 20000)  # Dataset (5000 cells
    , 20000 genes)
```

```
# Apply PCA
pca = PCA(n_components=50)
D_reduced = pca.fit_transForm(D)

# PerForm hierarchical clustering
Z = linkage(D_reduced, method='ward')
clusters = fcluster(Z, t=5, criterion='maxclust')

# Example ontology mapping (simplified)
ontology = {
    1: "Excitatory␣neuron",
    2: "Inhibitory␣neuron",
    3: "Astrocyte",
    4: "Oligodendrocyte",
    5: "Microglia"
}

# Annotate clusters
annotations = [ontology[cluster] For cluster in
    clusters]

# Output
annotated_D = np.column_stack((D, annotations))
```

Mathematical Details Let $D \in \mathbb{R}^{5000\times 20000}$ represent the dataset. PCA reduces this to $D' \in \mathbb{R}^{5000\times 50}$. Hierarchical clustering generates a dendrogram Z from which clusters are derived. Each cluster is mapped to a cell type based on the ontology.

Expected Output Each cell in the dataset is annotated with the cell type corresponding to its cluster. For example, if a query cell belongs to a cluster that matches astrocyte profiles, it is annotated as an astrocyte.

4 Summary and Conclusion

The surveyed research papers demonstrate the utility of using hierarchical ontology data for the annotation of single-cell RNA-seq (scRNA-seq) data. Each paper introduces unique methods that leverage cell type hierarchies to provide accurate and biologically meaningful annotations.

SingleR [*Aran, Looney, Liu, et al. 2019*], which uses a reference-based approach to annotate cells by comparing gene expression profiles to a curated reference dataset. The hierarchical structure of the reference dataset allows SingleR to provide multi-level annotations, ensuring that both specific and broader cell type categories are considered. This hierarchical approach improves the robustness and interpretability of the annotations, making SingleR a powerful tool for annotating scRNA-seq data with existing reference profiles.

SCINA [*Zhang, O'Flanagan, Chavez, et al. 2019*], a method that uses predefined marker gene sets to annotate cells. SCINA incorporates hierarchical ontology data to structure these marker gene sets, ensuring that annotations are consistent with known biological relationships. By using a probabilistic model, SCINA can provide both specific and broad annotations, reflecting the hierarchical structure of the cell ontology. This method is particularly useful for annotating cells with well-defined marker genes and can be customized for specific research needs.

The third paper [*Lake et al. 2016*] discusses a framework for integrating scRNA-seq data from multiple sources. This method aligns datasets using canonical correlation analysis and performs nearest neighbor search to identify similar cells. The use of hierarchical cell ontologies ensures that annotations are consistent across datasets and reflect known biological relationships. This approach is advantageous for creating comprehensive reference datasets that can be used to annotate novel scRNA-seq data, providing a robust foundation for accurate and consistent cell type annotations.

The paper focusing on Unsupervised Learning [*Shekhar et al. 2014*], explores unsupervised learning techniques for scRNA-seq data analysis. The proposed method uses hierarchical clustering and cell type ontologies to annotate cell clusters. By mapping clusters to a hierarchical ontology, the method provides both specific and broad annotations, enhancing the interpretability of the clustering results. This approach is particularly useful for discovering new cell types and states, as it does not rely on predefined labels and can uncover novel insights into cellular heterogeneity.

The fifth paper, focusing on Hierarchical Clustering [*Tasic et al. 2016*], introduces hierarchical clustering techniques combined with cell type ontologies to provide detailed annotations for complex tissues like the brain. This method ensures that annotations are biologically meaningful and consistent with known cellular hierarchies. By leveraging hierarchical clustering, the method can capture the intricate relationships between different cell types, making it robust for annotating scRNA-seq data from diverse and complex tissues.

Overall, each algorithm has its own advantages in being applied to novel scRNA data. SingleR and SCINA are particularly useful for leveraging existing biological knowledge through reference datasets and marker genes, respectively. The integration framework by Lake et al. is ideal for combining data from multiple sources, while the unsupervised learning approach by Shekhar et al. is well-suited for discovering new cell types without relying on predefined labels. The hierarchical clustering method by Tasic et al. is robust for annotating complex tissues and capturing intricate cellular relationships. By incorporating hierarchical ontology data, these methods ensure that cell type annotations are accurate, biologically meaningful, and consistent with known relationships, providing a robust framework for analyzing scRNA-seq data.

References

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